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continued on back cover

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Cover photo: Representatives of the two genera of present-day nautilus, *Allonautilus* and *Nautilus*; left, *Allonautilus scrobiculatus*; right, *Nautilus pompilius*. Evolutionary radiation of present-day *Nautilus* and *Allonautilus* (Bonacum *et al.* 77–93). Photo courtesy of W. Bruce Saunders.

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Inventory of Japanese sacoglossan opisthobranchs: Historical review, current records, and unresolved issues

Cynthia D. Trowbridge[1,*], Yayoi M. Hirano[2], and Yoshiaki J. Hirano[2]

[1] Oregon Institute of Marine Biology, Charleston, Oregon, U.S.A.

[2] Department of Biology, Graduate School of Science, Chiba University, Japan

Correspondence, C. D. Trowbridge: cdt@uoregon.edu

Abstract: In the past ~155 years, professional and amateur malacologists have recorded *ca.* 90 described species of sacoglossan opisthobranchs in ~25 genera on Japanese shores. In addition, there are at least 20 to 40 undescribed or unrecognized sacoglossans also recorded. The extraordinary species richness has been a source of admiration as well as vexation. Worldwide scientific excitement in this group was largely due to two pivotal discoveries by Japanese researchers: (1) the acquisition and retention of functional chloroplasts by the sacoglossan *Elysia atroviridis* Baba, 1955 and (2) the existence of extant populations of bivalved sacoglossans (initially *Tamanovalva limax* Kawaguti and Baba, 1959 and then related taxa). Eight of the nine sacoglossan families recognized by Jensen (1996, 2007) are represented in Japan. All the recognized sacoglossan genera are represented in Japan except: *Roburnella* Marcus, 1982; *Platyhedyle* Salvini-Plawen, 1973; *Gascoignella* Jensen, 1985; *Olea* Agersborg, 1923; *Limapontia* Johnston, 1836; and the Australian genera *Edenttellina* Gatliff and Gabriel, 1911 and *Midorigai* Burn, 1960. Taxonomic uncertainty has been caused by the absence of vouchers, incomplete and/or questionable descriptions, photographic misidentifications (books and internet), chronically unstable classification, and other scientific challenges; in particular, the small size, cryptic coloration, and patchy distribution of sacoglossans have contributed to limited collections of many species. Since 2000, we have collected, photographed, and preserved unusually large numbers of Japanese sacoglossans, including species traditionally considered rare by malacologists. Although it is premature to produce a comprehensive inventory of the Japanese sacoglossan fauna, we consider it necessary to describe explicitly the strengths and weaknesses of current information. This assessment should assist professional and amateur malacologists with future sacoglossan study, particularly in the areas of biogeography, phylogeny, and ecology.

Key words: biodiversity, ecology, kleptoplasty, Sacoglossa, species richness

Biogeographic patterns of species richness (*i.e.*, the number of species) exhibit broad-scale patterns along latitudinal gradients and among ocean basins; these patterns are robust across large numbers of taxonomic groups of plants and animals. Understanding the evolutionary and ecological processes producing and maintaining such patterns of biodiversity is a central objective of evolutionary ecology and phylogeny. The comprehensive investigation of species-rich areas or "hot-spots" will enhance our understanding of the role that biotic factors play in structuring communities. In marine communities, Indo-Pacific and Western Pacific shores are characterized by an extraordinary number of species.

Within the gastropod order (or clade) Sacoglossa, species richness values vary geographically by up to two orders of magnitude (see Jensen 2007). Low values are recorded for North Atlantic shores: 3 species for the Black Sea (Martynov *et al.* 2007), 5 for New England (Clark 1975), and 9 for the British Isles (Thompson 1988). In the NE Pacific region, there are at least 25 sacoglossan species from Alaska to the equator (Trowbridge 2002). Comparable information for the western Pacific, however, has been more problematic. There are at least 42 sacoglossan species on the Great Barrier Reef, 94 in the Marianas Islands, and *ca.* 80 in the Ryukyu Islands (Marshall and Willan 1999, Carlson and Hoff 2003, Ono 2004). Gosliner *et al.* (2008) recently reported *ca.* 107 sacoglossan species (including described and undescribed ones) for the Indo-Pacific region.

With the proliferation of local and regional web sites and photographic guides, it has become necessary to synthesize records for the western Pacific, particularly Japan which spans ~30° of latitude. When Baba (1937b) summarized opisthobranchs, there were only 9 species of sacoglossans recorded for Japan (Fig. 1). Spanning a range from 20° to 50°N latitude, Japanese shores are influenced by warm and cold-water currents that shape the opisthobranch fauna. Baba (1937b) suggested that the biogeographical break point of the warm- and cold-water fauna was around Akkeshi Bay on the Pacific shore of Hokkaido. Yet there have been only a few studies of sacoglossans from Hokkaido northward to the Kurile Islands or within the Sea of Japan (Baba 1935, Martynov

* Mailing address: P.O. Box 1995, Newport, Oregon 97365, U.S.A.

The rapid rate of sacoglossan species discovery and/or description has continued (Fig. 1), particularly due to the recent popularity of scuba diving, underwater photography, and internet slug sites. Ono (1999, 2004), Suzuki (2000), and Nakano (2004) have contributed immensely to the discovery of unknown sacoglossan species as well as the photographic documentation of described ones. Their efforts have been extremely valuable to amateur and professional malacologists. Ono's association with opisthobranch taxonomists at California Academy of Sciences has ensured that there are voucher specimens for most species and these are accessible to opisthobranch taxonomists. Ono's effect—particularly in conjunction with Gosliner (1995), Gosliner *et al.* (2008), Bolland (Okinawa Slug Site: http://www.rfbolland.com/okislugs/), and Rudman (Sea Slug Forum: http://seaslugforum.net/)—has been excellent. Ono has recorded a large number of Okinawan sacoglossans previously unknown from Japan.

There have been numerous other important malacologists (particularly Gosliner and Jensen) who formally described or redescribed sacoglossans that are now known to occur in Japan (Fig. 2). The actual records of collection of these species in Japan, however, are primarily from Ono (1999, 2004), Suzuki (2000), Nakano (2004), Bolland (Okinawa Slug Site), or others (on the Sea Slug Forum or various Japanese slug sites such as http://www.umiushi.info). For example, the first Japanese report of *Stiliger aureomarginatus* Jensen, 1993 was made by Suzuki (2000). Furthermore, Nakano (2004) published the first photograph of *Calliopaea pusillus* (Baba, 1959) (as *Stiliger pusillus* Baba, 1959); internet records may have preceded Nakano's printed record.

Authors

Trowbridge, Hirano, and Hirano have been collecting Japanese sacoglossans together since 2000. Voucher specimens have been deposited at Chiba University in the extensive Hirano opisthobranch collection. High-resolution photographic vouchers of species have been made during each collecting trip. Because our taxonomic work is currently in progress, we emphasize records pre-dating our research. Also, as many sacoglossans are undescribed (or perhaps just unrecognized) in Japan and elsewhere (Fig. 3), we consider the present paper to be an interim inventory—though an essential one—of Japanese species.

Shelled sacoglossans

Single-valved species

Although shelled sacoglossans are now known to be widely distributed and often abundant, these taxa have traditionally been considered rare. On Japanese shores, the single-shelled species (the oxynoids and volvatellids) have been known for many decades. Prof. Tadashige Habe described

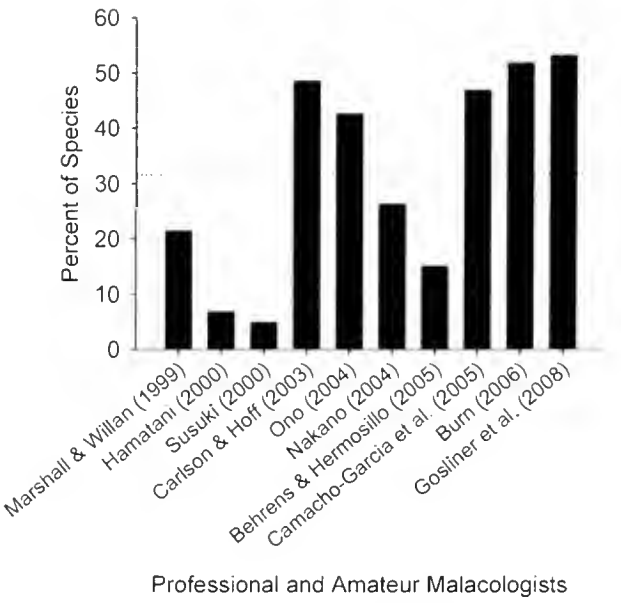

Figure 3. The estimated percentage of sacoglossan species that are unidentified—either undescribed or unrecognized—in several recent field or literature surveys of opisthobranchs. As several authors present taxa as species complexes, these values are inherently estimates. The dotted horizontal line represents the mean value of unidentified species.

Volvatella kawamurai Habe, 1946 and provided the first report of *Oxynoe* in Japan (Habe 1946, 1949). As mentioned in the previous section, Hamatani described four shelled species (*Ascobulla japonica*, *Volvatella ayakii*, *Volvatella viridis*, and *Oxynoe kabirensis*) and Ichikawa described one (*Volvatella angeliniana* Ichikawa, 1993). Kitao (1979) published descriptions of the veliger larvae of a Japanese *Volvatella* and *Oxynoe*.

Bivalved species

In terms of bivalved sacoglossans (Juliidae), Kuroda (1935a, 1935b) first reported shells of *Julia exquisita* (Gould, 1862); however, these two early reports listed the species as a member of the bivalve family Ungulinidae. It was only when the living bivalved sacoglossans were discovered that they were reassigned to the opisthobranch gastropods: Kuroda and Habe (1951) described *Julia japonica*, a small but frequent species. Extant populations of Juliidae and aspects of their intriguing biology were described by Kawaguti (1959, 1982), Kawaguti and Baba (1959), Kawaguti and Yamasu (1959, 1960a, 1960b, 1960c, 1961a, 1961b, 1962, 1966a, 1966b, 1967), Baba (1961a, 1961b), Kitao (1978), and Yamasu (1997). The records of *Tamanovalva limax* (called *Berthelinia limax* by many but not all malacologists) and subsequently *Julia* spp. (Kawaguti and Yamasu 1962, 1982, Yamasu 1968, 1969, Kawaguti 1981) stimulated the international research community immensely. For example, Cox and Rees (1960) published a *Nature* commentary of the paper of Kawaguti and Baba (1959), highlighting the discovery of the live specimens

and the recognition that these bivalved species are actually gastropods. Soon reports of bivalved sacoglossans were made from Hawaii (Kay 1962a, 1962b, 1968), California (Keen 1960, Keen and Smith 1961, Smith 1961), Jamaica (Edmunds 1962, 1963), Australia (Burn 1960a, 1960b, 1960c), and India (Rao 1965, Ganapati and Sarma 1972, Sarma 1975).

Although some researchers have suggested referring to all Pacific *Julia* specimens as *Julia exquisita* (Gould, 1862), this advice was based on the erroneous assumptions that specimen numbers were insufficient for study and that detailed anatomical work had not yet been done. We take the conservative approach of retaining the described names, particularly given our knowledge of extensive unpublished Japanese work on this group (discussed below).

Anatomy, development, and field associations

In addition to stimulating international research, Kawaguti and Yamasu (together and individually) have produced an entire series of highly detailed papers on the internal anatomy of *Tamanovalva limax*, *Julia japonica*, and others. These morphological papers—with the addition of ones by Kawaguti (1941, 1968), Kawaguti *et al.* (1965), Baba and Hamatani (1970a, 1970b), Baba (1986), Adachi (1991), Hirose (2005), and Hirano *et al.* (2006b, 2007c, 2008a, 2008b, 2009, 2010a, 2010b)—are the only detailed accounts of Japanese sacoglossan anatomy. Thus, the extraordinary diversity of sacoglossans in Japan has not yet been accompanied by extensive anatomical work. There is a pressing need for anatomical and developmental work of Japanese species comparable to the excellent standard set by Kawaguti, Yamasu, and colleagues.

From 1986 to 1991, at least six students at the University of Ryukyus in Okinawa investigated sacoglossans (T. Iseto, pers. comm.). For example, the development of *Julia* and *Tamanovalva* were studied by Sato (1986), Chibana (1987), and Iseto (1996); the comparative morphology of Juliidae was investigated by Mizofuchi (1987, 1990) and Murayama (1989). Intriguingly, Mizofuchi (1990) compared *Berthelinia* and 5 *Julia* species—1 species more than reported by more recent amateur or professional malacologists. To our knowledge, the wealth of Okinawan research on these bivalved sacoglossans has not yet been published within the Japanese or international literature with the exception of tantalizing abstracts (Mizofuchi and Yamasu 1987, Yamasu and Adachi 1990). However, Iseto (pers. comm.) indicated he would publish a synthesis of those.

Our group's success in locating shelled sacoglossans in Okinawa was a direct result of the valuable and detailed collection advice of Prof. Yamasu shortly before our survey program started. For example, in six brief visits to Okinawa from July 2002 to March 2005, we found Volvatellidae (5 *Volvatella* spp., 49 specimens), Oxynoidae (3 species, 37 specimens), and Juliidae (4 species, 8 specimens). The perception that these species are unusual or rare is not strictly true. In fact, Mizofuchi and Yamasu (1987: 1112) reported that "Several hundred living specimens of genus *Julia* were collected ... in 1986 and 1987." Yamashita *et al.* (1998) found six live *Julia* specimens on a single day on Tsunoshima Island, NW Yamaguchi Prefecture, Japan. Finally, Kawaguti and Yamasu (1982) collected 13 live specimens of *J. japonica* at Tsunoshima and *ca.* 20 from Ishigaki Island in Okinawa.

Self-fertilization

The report of self-fertilization in a bivalved sacoglossan (Kawaguti and Yamasu 1961a) also intrigued malacologists. Although all sacoglossans are hermaphroditic, self-fertilization has been described in only two other cases of the other hundreds of species worldwide. The Atlantic *Berthelinia caribbea* Edmunds, 1963 produces offspring without mating (Grahame 1969) and the NE Pacific *Alderia willowi* Krug *et al.*, 2007 does as well (Smolensky *et al.* 2009). The latter study explored the costs of mating, particularly given the species' hypodermic insemination, in an elegant series of controlled experiments.

Kleptoplasty or chloroplast retention

Initial discovery

One of the major contributions of Japanese sacoglossan researchers is the discovery that ingested algal chloroplasts were retained within sacoglossan digestive diverticulae. This phenomenon—now called kleptoplasty—was described by Kawaguti and Yamasu (1965) in reference to *Elysia atroviridis* Baba, 1955 associated with the green alga *Codium fragile*. Kawaguti (1941) and Kawaguti *et al.* (1965) also investigated *Plakobranchus* but the system was not understood as well as the *Elysia* one (*e.g.*, retained algal chloroplasts were interpreted as unicellular algae). Extensive work (well-reviewed elsewhere) was subsequently conducted by European workers on the ecological analog of *E. atroviridis*, namely *Elysia viridis*, of NE Atlantic shores, particularly the functionality of the retained chloroplasts and their role in the energetics of the slugs. Despite the discovery of the concept of kleptoplasty, researchers have not yet characterized the frequency with which it occurs within the diverse sacoglossan group on Japanese shores.

Pulse amplitude modulated fluorometry (PAM)

A new method, namely the use of PAM to characterize the functionality of ingested chloroplasts, shows great potential in characterizing Japanese species. Sue Williams (2000a, 2000b) used PAM and mass spectrometry to investigate the photobiology of Australasian sacoglossans for her

Ph.D. research at University of Western Australia at Perth; the methodology was subsequently used by Evertsen *et al.* (2007), Grzymbowski *et al.* (2007), and Händeler *et al.* (2009, 2010) for a suite of Indo-Pacific and Mediterranean species, several of which also occur in Japan. This methodology was recently employed by Yamamoto *et al.* (2008, 2009) to investigate Japanese sacoglossans; they found photosynthetically active chlorophyll in *ca.* 8-10 species in five families. This research group is continuing their work on functional kleptoplasty in Japanese sacoglossan assemblages (Yamamoto, pers. comm.).

Molecular approaches

Several research groups have started using molecular techniques to identify the retained algal chloroplasts within sacoglossan tissues. The methodology, first described by Curtis *et al.* (2006, 2007) for Florida sacoglossans, is being employed by the research groups of Dr. Tadashi Maruyama and Prof. Euichi Hirose in Japan. For example, for his Ph.D. research, Taro Maeda is investigating chloroplast retention in *Plakobranchus ocellatus* van Hasselt, 1824 (*sensu lato*) and using molecular tools to identify the algal hosts (Maeda *et al.* 2009). Maeda (2009) discussed whether genes involved in photosynthesis are transferred from algal host to sacoglossans.

Ecological research

Field populations

Numerous researchers have recorded algal host associations of sacoglossan herbivores on the shore. However, quantitative ecological work rigorously investigating feeding preferences, diet breadth, and host-use has only recently been initiated (*e.g.*, Usuki 1977, Hirano and Hirano 1991, Shimadu 2005, Shimadu *et al.* 2006, Hirano *et al.* 2007b, Kumagai *et al.* 2008, Trowbridge *et al.* 2005, 2006, 2008a, 2008b, 2009a, 2009b, 2009c, 2010a, 2011, Kumagai 2009). Population studies of Japanese sacoglossans are meager (but see Usuki 1977, Hirose *et al.* 2003, Shimadu 2005, Shimadu *et al.* 2006, Trowbridge *et al.* 2008a, 2008b, 2009a, 2009b, 2010a, 2010b, 2010c, 2011, and Kumagai 2009 for notable exceptions). Usuki's research was particularly noteworthy because it was both quantitative and comparative; Usuki compared the ecology of *Ercolania boodleae* (Baba, 1938) to its European and North American analogs feeding on filamentous green algae. Quantitative life-history attributes of *Tamanovalva limax*, including life span, were also characterized by Kawaguti and Yamasu (1960b).

Reproductive ecology

Detailed descriptions of spawn masses, egg and capsule sizes, and veliger larvae have been made by Baba *et al.* (1956), Hamatani (1960, 1963, 1967), and Kitao (1979). Characterization of spawn masses, embryos, and larval type (planktotrophic and lecithotrophic) of all Japanese sacoglossans is currently in progress by the authors of the present paper. Quantitative larval ecology of Japanese sacoglossans has not yet been endeavored despite the diversity of species. Given that the majority of the sacoglossans have long-lived planktonic larvae, the paucity of study is understandable; few such species have been cultured through their life cycles. Understanding the breadth of larval choice among sympatric host and non-host algae would enable us evaluate how frequently sacoglossans are extreme specialists (*e.g.*, monophagous herbivores) vs. less stenophagous ones. Larval work on the European *Elysia viridis* demonstrated (Trowbridge and Todd 2001) that sacoglossans can have much broader metamorphic cues than traditionally presumed: the adult food is not the sole metamorphic trigger, in contrast to what has traditionally been presumed.

Diet breadth

Recent work on *Codium*-feeding sacoglossans on Honshu demonstrated that the high diversity of *Codium* spp. (*ca.* 20) is attacked by slugs. Monophagy (*i.e.*, extreme specialization) was not found in the high-diversity temperate systems (Trowbridge *et al.* 2009a) or in subtropical Okinawan ones (Trowbridge *et al.* 2010c). The generality of these results to the *Caulerpa*-feeding species remains to be seen but is under current investigation by the authors.

Current topics

Three fields of sacoglossan ecology currently being investigated are parasites, competitors, and predators. Kosuke Sudo at Chiba University has been characterizing the frequency and identity of parasites. Sudo (2005, 2007) and Sudo *et al.* (2005, 2007, 2008, 2009, 2010, 2011) described splanchnotrophid copepods and turbellarian flatworms that parasitize four and six species of sacoglossans, respectively, on the Pacific shores of Honshu. Additional parasite records have been noted for Okinawa and Kyushu (Sudo, pers. comm.). Sudo repeatedly found both types of parasites, sometimes abundantly, from at least a few to several sacoglossan species (Sudo *et al.* 2010, 2011). Sudo's research shows that investigating parasite-host interactions merits further study as a generally overlooked, but potentially important, interaction.

Trowbridge *et al.* (2009a) evaluated the nature of interspecific interactions of sympatric sacoglossan species—competition, facilitation, or no interactions. Field observations and laboratory experiments indicated there was no evidence of competition or facilitation among coexisting species on *Codium* in Sagami Bay, Honshu.

Finally, in Okinawa, Nakano *et al.* (2007) have documented the feeding of *Gymnodoris* Stimpson, 1855 on other opisthobranchs: sacoglossans are among the prey species. Based

on this Japanese work and earlier research on NE Pacific and NE Atlantic shores (Trowbridge 1994 and unpubl. data), sacoglossan predators include some fishes, birds, crabs, nudibranchs, and mites. Because sacoglossans dwell within marine communities, there is real need to understand the interspecific and intraspecific interactions of the species rather than just the sacoglossans (in isolation) or the sacoglossan-algal interactions (without other interacting species) as traditionally investigated.

KNOWN TAXA

Classification systems

Baba (1937b: 195) remarked that: "The classification and nomenclature of the Opisthobranchia are serious problems still not satisfactorily resolved. Subsequent investigations therefore will render it necessary to alter some of the accounts of the species, genera or even families contained in this paper." These words accurately reflect the taxonomic uncertainty still plaguing sacoglossan researchers today. Jensen (1996) has shown the validity of sacoglossan families. Recent molecular work [including Bass and Karl (2006), Händeler and Wägele (2007), Händeler *et al.* (2009), Maeda *et al.* (2010), and Krug *et al.* (in progress)] have partly confirmed the validity of some families and genera. Our present account does not establish new taxonomic considerations but rather will bridge the gap between species' initial records on Japanese shores and the currently used names and systematic arrangement.

Families, genera, and species

All 3 families of suborder Oxynoacea are represented in Japan: Volvatellidae, Juliidae, Oxynoidea. Of suborder Plakobranchacea, 5 of the 6 families are represented: Plakobranchidae, Bosellidae, Polybranchiidae, Hermaeidae, and Limapontiidae but not Platyhedylidae.

Of the 31 sacoglossan genera recognized by Jensen (1996), 25 have been reported from Japan (Table 1). The only sacoglossan genera not yet reported from Japan are *Roburnella* Marcus, 1982 (Oxynoidae); *Platyhedyle* Salvini-Plawen, 1973 and *Gascoignella* Jensen, 1985 (Platyhedylidae); *Olea* Agersborg, 1923 and *Limapontia* Johnston, 1836 (Limapontiidae); and *Edenttellina* Gatliff and Gabriel, 1911 and *Midorigai* Burn, 1960 (Juliidae). However, the validity of several genera has been questioned so the precise genus count is controversial.

The number of described sacoglossan species recorded from Japanese shores is about 90 (Table 1) while the number of recognized species exceeds 100; the total number—including undescribed species recognized by Carlson and Hoff (2003), Ono (2004, 2005), Gosliner *et al.* (2008), and our research group—will probably be *ca.* 125-140 sacoglossan species. The degree of uncertainty is a direct result of the high percentage (*ca.* 32%) of unidentified sacoglossan species (Fig. 3), either undescribed cryptic species or unrecognized ones. By unrecognized species, we refer to those species for which authors have not assigned a species name. As many of the Japanese sacoglossans are broadly distributed Indo-Pacific species, we adopt the conservative approach of comparing our species to all congeners described (worldwide) before presuming our species are local endemics and describing them as such. Several of Baba's described species were subsequently synonymized with Indo-Pacific congeners. Given the challenges of finding the full citations of type descriptions of sacoglossans, many professional and amateur malacologists publish their records as unidentified species.

Malacologists are still on the steep part of the species and genus accumulation curves (Fig. 1). The rapid rate of species discovery and/or description has continued, particularly due to the recent popularity of scuba diving, underwater photography, and internet slug sites. Although photographic identifications often are challenging, the misidentifications made are understandable given the difficulty in distinguishing some species.

THE CHALLENGES

There is still considerable taxonomic controversy regarding several family, genus, and species names. At the family level, we follow the classification system of Jensen (1996) while acknowledging the existence of alternate systems currently in use on the *Sea Slug Forum* (Australian Museum) and by Valdés and Bouchet in Bouchet and Rocroi (2005). Until molecular studies fully elucidate the relationships of genera, there will be ongoing disagreement among colleagues about sacoglossan classification. Maeda (2007) investigated the molecular phylogeny of the Sacoglossa for his M.Sc. thesis. This work (Maeda *et al.* 2006, 2010) complements ongoing research by two other research groups (Patrick Krug at California State University in Los Angeles; Heike Wägele at the University of Bonn in Germany).

At the genus level, there continues to be a lack of consensus about the status of: (1) *Pattyclaya* Marcus, 1982 and *Elysiella* Verrill, 1872 and (2) *Tamanovalva, Berthelinia, Edenttellina,* and *Midorigai*. Molecular and cladistic evidence supports the elimination of *Elysiella* as the species fall within the clade of *Elysia* (Gosliner 1995, Bass and Karl 2006, Händeler and Wägele 2007, Händeler *et al.* 2009); comparable information is needed for *Pattyclaya*. With respect to the bivalved taxa, continued uncertainty or disagreement includes the proper genus name for *Tamanovalva limax* and *Berthelinia schlumbergeri* Dautzenberg, 1895 (*e.g.*, Baba 1961a, 1961b, Rudman 2001e, 2002b). Opisthobranch taxonomists and other researchers are still divided on the use of the same genus name (*Berthelinia* Crosse, 1875) for extinct and extant species

Table 1. List of all described sacoglossan species recorded from Japanese shores. Citations of taxonomic authorities are listed in the literature-cited section. Notes are summarized in Appendix 1.

Family	Species	Authority	Notes
Volvatellidae			
	Ascobulla japonica	(Hamatani, 1969)	
	Volvatella angeliniana	Ichikawa, 1993	
	Volvatella ayakii	Hamatani, 1972	
	Volvatella kawamurai	Habe, 1946	
	Volvatella vigourouxi	(Montrouzier in Souverbie, 1861)	1
	Volvatella viridis	Hamatani, 1976	2
Oxynoidae			
	Lobiger souverbii	Fisher, 1856	3
	Lobiger viridis	Pease, 1863	4
	Oxynoe kabirensis	Hamatani, 1980	
	Oxynoe viridis	(Pease, 1861)	
Juliidae			
	Berthelinia schlumbergeri	Dautzenberg, 1895	5
	Julia exquisita	(Gould, 1862)	
	Julia japonica	Kuroda and Habe, 1951	6
	Julia mishimaensis	Kawaguti and Yamasu, 1982	
	Julia zebra	Kawaguti, 1981	7
	Tamanovalva limax	Kawaguti and Baba, 1959	8
Plakobranchidae			
	Elysia abei	Baba, 1955	9
	Elysia amakusana	Baba, 1955	10
	Elysia atroviridis	Baba, 1955	11
	Elysia babai	Pruvot-Fol, 1946	12
	Elysia bennettae	Thompson, 1973	13
	Elysia cf. *tomentosa*	Jensen, 1993	14
	Elysia flavipunctata	Ichikawa, 1993	15
	Elysia hamatanii	Baba, 1957	
	Elysia hirasei	Baba, 1955	16
	Elysia japonica	Eliot, 1913	17
	Elysia lobata	Gould, 1852	18
	Elysia mercieri	(Pruvot-Fol, 1930)	
	Elysia minima	Ichikawa, 1993	19
	Elysia nigrocapitata	Baba, 1957	
	Elysia obtusa	Baba, 1938	20
	Elysia ornata	(Swainson, 1840)	21
	Elysia pusilla	(Bergh, 1872)	22
	Elysia rufescens	(Pease, 1871)	23
	Elysia setoensis	Hamatani, 1968	24
	Elysia sugashimae	Baba, 1955	25
	Elysia thompsoni	Jensen, 1993	26
	Elysia trisinuata	Baba, 1949	27
	Elysia yaeyamana	Baba, 1936	28
	Elysiobranchus ryukyuensis	Ichikawa, 1993	29
	Pattyclaya arena	Carlson and Hoff, 1977	30
	Plakobranchus ocellatus	van Hasselt, 1824	31
	Thuridilla albopustulosa	Gosliner, 1995	
	Thuridilla bayeri	(Marcus, 1965)	32
	Thuridilla carlsoni	Gosliner, 1995	

Table 1. (continued)

Family	Species	Authority	Notes
	Thuridilla flavomaculata	Gosliner, 1995	
	Thuridilla gracilis	(Risbec, 1928)	33
	Thuridilla hoffae	Gosliner, 1995	
	Thuridilla kathae	Gosliner, 1995	
	Thuridilla livida	(Baba, 1955)	
	Thuridilla splendens	(Baba, 1949)	
	Thuridilla undula	Gosliner, 1995	
	Thuridilla vatae	(Risbec, 1928)	
Polybranchiidae/Caliphyllidae			
	Cyerce kikutarobabai	Hamatani, 1976	
	Cyerce nigricans	(Pease, 1866)	
	Mourgona osumi	Hamatani, 1994	
	Polybranchia orientalis	(Kelaart, 1858)	
	Sohgenia palauensis	Hamatani, 1991	
Limapontiidae			
	Alderia modesta	(Lovén, 1844)	34
	Alderiopsis nigra	(Baba, 1937)	34
	Aplysiopsis minor	(Baba, 1959)	
	Aplysiopsis nigra	(Baba, 1949)	
	Aplysiopsis orientalis	(Baba, 1949)	
	Aplysiopsis toyamana	(Baba, 1959)	
	Calliopaea pusillus	(Baba, 1959)	35
	Costasiella formicaria	(Baba, 1959)	
	Costasiella iridophora	Ichikawa, 1993	
	Costasiella kuroshimae	Ichikawa, 1993	
	Costasiella paweli	Ichikawa, 1993	
	Costasiella rubrolineata	Ichikawa, 1993	
	Costasiella usagi	Ichikawa, 1993	
	Costasiella vegae	Ichikawa, 1993	36
	Ercolania kencolesi	Grzymbowski *et al.*, 2007	37
	Ercolania boodleae	(Baba, 1938)	
	Ercolania coerulea	(Trinchese, 1893)*	
	Ercolania subviridis	(Baba, 1959)	38
	Hermaea noto	(Baba, 1959)	39
	Hermaea vancouverensis	O'Donoghue, 1924	40
	Hermaea wrangeliae	(Ichikawa, 1993)	41
	Hermaea zosterae	(Baba, 1959)	39
	Placida cremoniana	(Trinchese, 1893)*	
	Placida dagulareusis	Jensen, 1990	
	Placida sp. (*sensu* Baba 1986)**	Baba, 1986	42
	Stiliger aureomarginatus	Jensen, 1993	
	Stiliger berghi	Baba, 1937	43
	Stiliger ornatus	Ehrenberg, 1828	
	Stiliger smaragdinus	Baba, 1949	44

* not 1892 as stated by many authors (books, papers, and internet)

** described but not named

because the hypothesis of convergent shell shape cannot be refuted.

At the species level, there persists the concern of whether certain taxa are species complexes or single, phenotypically variable species. Problematic "species" include *Placida dendritica* reported for Japan (Baba 1937b), redescribed by Marcus (1982), and then again by Baba (1986). Bleakney (1989) prematurely synonymized many *Placida* spp. from

Table 2. List of undescribed but recognized sacoglossan species recorded from Japanese shores. Our unpublished data are preliminary estimates.

Families	Genera	Bolland (*Okinawa Slug Site*)	Hamatani (2000)	Ono (2004)	Nakano (2004)	Hirano *et al.* (unpubl. data)
Volvatellidae	*Volvatella*	2				1-2?
Bosellidae	*Bosellia*	1		1	1	1
Plakobranchidae	*Elysia*			12	7	11-12?
	Pattyclaya		1			
	Plakobranchus		1			4-5
	Thuridilla			1		
Polybranchiidae/Caliphyllidae	*Caliphylla*					1
	Cyerce	2		5	4	
	Polybranchia					2
	Sohgenia			1		1
Limapontiidae	*Aplysiopsis*	1				
	Costasiella	3		3		2-3
	Ercolania	2		7	1	7-8?
	Hermaea	4		3	1	
	Placida	1				1-2
	Total	16	2	33	14	31-37

temperate shores around the world based almost exclusively on radular tooth morphology but did not present any reproductive comparisons or other morphological features. Hirano *et al.* (2006a, 2006c) clarified several of the cryptic Japanese species attributed to the name *P. dendritica*. Shimadu (2005), Shimadu *et al.* (2006), Kumagai *et al.* (2008), and Kumagai (2009) reported ecological and morphological work on *Placida* sp. (*sensu* Baba 1986). In fact, Gosliner *et al.* (2008: 72) noted that "Many members of this genus have been lumped under the name *Placida dendritica*, but different populations have distinctive branching patterns of the digestive gland and probably represent a complex of distinct species." Martynov (2006) also illustrated two "forms" of *P. dendritica* with different branching patterns on Russian shores; he suggested they may be seasonal ecotypes (summer vs. winter forms) or separate species (Martynov, pers. comm.).

A second example is the long-term controversy regarding the number of *Plakobranchus* spp. occurring in Okinawan waters as well as circum-tropical regions worldwide. *Placobranchus guttatus* was described by Stimpson (1855) but Baba (1936) synonymized this species with *P. ocellatus*. Yamasu and Adachi (1990), Adachi (1991), Yamasu (1997), Hirano *et al.* (2005), and Ono (2005) have all remarked on the 4-5 types or morphs of *P. ocellatus* in Okinawa and hypothesized that the taxon is actually a complex of sibling species. Furthermore, Hamatani (2000) recognized two species. Based on external coloration (authors' unpubl. data on >1000 specimens from Okinawa), population dynamics, development, and genetic differences (P. Krug, pers. comm.), there are multiple distinct species being recognized under the name *P. ocellatus* in Japan. The species boundaries have not yet been fully established but are currently in progress.

As the Japanese fauna becomes more studied, some of the species originally considered to be endemic to Japan are being synonymized with other, particularly Indo-Pacific species. Recent examples include (1) *Elysia kushimotoensis* Baba, 1957, now considered to be *E. rufescens* (Pease, 1871) and (2) *Elysia tokarensis* Baba, 1957, now considered to be *E. lobata* Gould, 1852 (*e.g.*, Nakano 2004, Ono 2004). Both of these synonymies were based on photographs with no published records of anatomical comparisons or acknowledgment of detailed published descriptions of the Japanese species (*e.g.*, Kitao 1977). Another example is the suggested or stated synonymy of *Elysia obtusa* Baba, 1938 and *Elysia flava* Verrill, 1901 (*e.g.*, Rudman 2001a, Gosliner *et al.* 2008, and many internet sites). Although the first two suggested synonymies are seemingly not controversial, the third one is problematic as it involves a Pacific and an Atlantic species. With scientists' recent focus on introduced species, are we prematurely considering species to be conspecific based on external similarity alone despite known biogeographical boundaries? [For example, see discussion on *Sea Slug Forum* about *Bosellia* from the western Pacific (Rudman 2004)]. Although photographic comparisons and informal, online discussions are informative, they are not sufficient, by themselves, for taxonomic synonymies. Morphological, reproductive, and genetic evidence should still be rigorously evaluated particularly in the high-diversity, but largely unexplored, group.

TYPE DESCRIPTIONS

Given the current journal publication convention not to list the citation details of type descriptions, the valuable details necessary to verify sacoglossan species are becoming extremely difficult to locate. In larger groups such as nudibranchs, researchers have compiled and published bibliographies and full citations of type descriptions (*e.g.*, McDonald 2009a, 2009b). To ensure that such crucial sacoglossan information is preserved for future malacologists, we have included the citations of the original descriptions of Japanese sacoglossans (Appendix 2).

CONCLUSIONS

There is a wealth of information in Japanese that is relatively inaccessible to and/or unrecognized by most non-Japanese malacologists. For example, many of the journals are not electronically indexed so citations do not appear in electronic databases used in the U.S., Europe, etc. Our brief review of the scope and details of Japanese contributions should facilitate and enhance the study of sacoglossan research worldwide. There are pressing needs to investigate (1) shelled sacoglossan species and both *Ercolania* and *Placida* species complexes; (2) the population ecology and ecological role of sacoglossans; (3) the role of genetics and development in determining host choice; and (4) speciation on the high-diversity but taxonomically challenging shores of Japan and environs.

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Appendix 1. Explanatory notes relating to described sacoglossan species listed in Table 1.

#	Notes
1	Rudman (2002f)
2	Rudman (2002e)
3	*Lobiger souverbii* is the correct spelling and this species is synonymous with *L. sagamiensis* Baba, 1952 (Baba 1974, Rudman 2000c, 2001c).
4	Rudman (2001c)
5	Genus name controversial (Rudman 2002b).
6	May be synonymous with *J. zebra*; but see Kitao (1978).
7	May be synonymous with *J. thecapliora* Carpenter, 1857 (Pittman 2001).
8	Genus name controversial (Rudman 2001e, 2002b).
9	The questionable distinction between this species and *E. amakusana* was acknowledged in its description (Baba 1955a); both are now considered color morphs of *E. abei* (Rudman 2002g).
10	The questionable distinction between this species and *E. abei* was acknowledged in its description (Baba, 1955b); both are now considered color morphs of *E. abei* (Rudman 2002g).
11	Genetically indistinguishable from *E. setoensis* (A. Bass, pers. comm.; P. Krug, pers. comm., different specimens).
12	Never recorded since its description: Baba (1936) reported *E. viridis* from Okinawa but the radular teeth are extremely different from those of the European species; consequently, Pruvot-Fol renamed the Okinawan species *E. babai* Pruvot-Fol, 1946. However, there have been no subsequent records of this species so its identity is unclear.
13	Controversial about this species' identity and features differentiating it from *Elysia yaeyamana* (Carlson and Hoff 1978, 2003, Marshall and Willan 1999; Trowbridge, pers. obs.)
14	Species complex (Rudman 2001d, Ono 2004, P. Krug, unpubl. data); see Kitao (1976) for first published Japanese record.
15	Never recorded since its description.
16	Species recorded as *E. viridis* by Hirasé (1927), Baba (1949), Taki (1951); it then was redescribed as *E. hirasei* (Baba 1955a); also see (Rudman 2005c, 2009).
17	Type description too vague, no type locality information, and no voucher; Rudman suggests we abandon the species name (Rudman 2002a, 2002g).
18	No published anatomical comparisons made to substantiate name change from *E. tokarensis* Baba, 1957 (see Rudman 2001b, Nakano 2004); for anatomy of Japanese specimens, see Baba (1957) and Kitao (1977).
19	Never reported since its description though we may have re-discovered it in April 2010.
20	May be synonymous with *E. flava* (Rudman 2001a, Gosliner *et al.* 2008) but controversial.
21	May be synonymous with *E. marginata* and *E. grandifolia* (see Rudman 1999b, 2001b; Jensen 2001b) or may be a complex of cryptic species; molecular evidence supports the latter (P. Krug, unpubl. data).
22	*Elysiella* synonymized with *Elysia* (Gosliner 1995, Bass and Karl 2006, Händeler and Wägele 2007, Händeler *et al.* 2009, P. Krug, unpubl. data).
23	No external or internal anatomical comparisons made to support name change from *E. kushimotoensis* Baba, 1957 (see Rudman 1999a, 2002d).
24	Genetically indistinguishable from *E. atroviridis* (P. Krug, pers. comm.) but is morphologically distinct.
25	Synonymous with *E. trisinuata*: intraspecific variation in tooth shape (Trowbridge *et al.* 2006).
26	Ono (2004); Hirano *et al.* (unpubl. data)
27	Synonymous with *E. sugashimae*: intraspecific variation in tooth shape (Trowbridge *et al.* 2006).
28	Unclear about this species' identity and features differentiating it from *Elysia bennettae* (Marshall and Willan 1999, Carlson and Hoff 2003, Trowbridge, pers. obs.).
29	Never recorded since its description; genus not recognized by all opisthobranch taxonomists.
30	May be *Elysia arena*; validity of genus name is controversial (Rudman 2006a).
31	Species complex: at least 4-5 color morphs in Japan with at least 3 distinct species (Yamasu and Adachi 1990, Adachi 1991, Ono 2004, 2005, Trowbridge *et al.*, unpubl. data, P. Krug, unpubl. data).
32	Controversy of synonym (Rudman 2000a).
33	Controversy of synonym (Rudman 2000b).
34	Baba (1937a, 1968) described and redescribed the species *Alderia/Alderiopsis nigra*. Nishina found some similar specimens; molecular evidence (P. Krug, pers. comm.) indicates these were *Alderia modesta*. Russian specimens of *A. modesta* have also been reported (Chernyshev and Chaban 2005, Rudman 2005a). However, *A. nigra* was recently discovered elsewhere and CDT confirmed the specimens; therefore, we consider Baba's specimens and Nishina's specimens are separate species.

Appendix 1. (Continued)

#	Notes
35	Genus name uncertain. Described as *Stiliger* by Baba (1959). Baba and Hamatani (1970a) suggested species belonged to *Calliopaea*; no longer considered *Stiliger pusillus* as published by many amateurs.
36	Rudman (2006b)
37	Hirano *et al.* (2006d, 2007c, unpubl. research); also reported by Carlson and Hoff (2003).
38	Hirano *et al.* (unpubl. research), Rudman (2005b); *Ercolania subviridis* (Baba, 1959) may be the same as *Ercolania varians* (Eliot, 1904) reported by Carlson and Hoff (2003) in the Marianas. Eliot's type specimens have not been located.
39	*Hermaea noto* may be synonymous with *H. zosterae* (Jensen 2000, Rudman 2002c) but the tooth counts are extremely different, indicative of separate species. *Hermaea noto* has 10 ascending + 38 descending teeth whereas *H. zosterae* has 6 ascending + 13 descending ones.
40	Genus name and species identity uncertain: *Stiliger akkeshiensis* Baba, 1935 was described from Akkeshi Bay, Hokkaido. It appears that *S. akkeshiensis* refers to *Hermaea vancouverensis* O'Donoghue (1924) described from NE Pacific shores (Hirano *et al.* 2008a, 2008b). The Russian specimens recorded by Martynov for the Kuriles have been confirmed to be *H. vancouverensis* (see Rudman 2005d).
41	Original genus assignment (*Aplysiopsis*) incorrect; no vouchers and insufficient description (Trowbridge *et al.* 2009b).
42	See Hirano *et al.* (2006a, 2006b, 2006c).
43	Genus name uncertain: probably belongs to undescribed genus (Baba and Hamatani 1970a) or to *Hermaea* (Hirano *et al.* 2008a).
44	Genus name uncertain: probably belongs to undescribed genus (Baba and Hamatani 1970a, Jensen 2001a).

Appendix 2. Citations of type descriptions of Japanese sacoglossans. * denotes Japanese descriptions of species subsequently synonymized with Indo-Pacific ones.

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Allozyme analysis of Japanese *Semisulcospira* species (Gastropoda: Pleuroceridae) reveals that Lake Biwa endemic species are not monophyletic*

Satoshi Kamiya[1], Masanori Shimamoto[2†], and Takashi Hashimoto[3]

[1] Institute of Geology and Paleontology, Graduate School of Science, Tohoku University, 6-3 Aoba, Aoba-ku, Sendai 980-8578, Japan

[2] The Tohoku University Museum, 6-3 Aoba, Aoba-ku, Sendai 980-8578, Japan

[3] Nihon M&A Center Inc., 1-8-3 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan

Correspondence, Masanori Shimamoto: meteoroid@ma.mni.ne.jp

Abstract: Lake Biwa (Shiga Prefecture, Japan) is an ancient lake with many endemic species. In particular, 15 extant endemic species of the genus *Semisulcospira* (Caenogastropoda: Pleuroceridae) have attracted attention because they may represent a single species flock. Although many taxonomic studies have been conducted on these Lake Biwa species and on non-Biwa species of the genus, their genetic relationships remain unresolved. We use allozyme analysis to investigate the genetic differentiation of 12 species of the genus *Semisulcospira*, including nine Lake Biwa endemics: *S.* (*Biwamelania*) *arenicola* Watanabe and Nishino, 1995, *S.* (*B.*) *decipiens* (Westerlund, 1883), *S.* (*B.*) *fluvialis* Watanabe and Nishino, 1995, *S.* (*B.*) *multigranosa* (Böttger, 1886), *S.* (*B.*) *nakasekoae* Kuroda, 1929, *S.* (*B.*) *ourense* Watanabe and Nishino, 1995, *S.* (*B.*) *fuscata* Watanabe and Nishino, 1995, *S.* (*B.*) *niponica* (Smith, 1876), and *S.* (*B.*) *habei* Davis, 1969, and three non-Biwa species: *S.* (*S.*) *libertina* (Gould, 1859), *S.* (*S.*) *reiniana* (Brot, 1877), and *S.* (*S.*) *kurodai* Kajiyama and Habe, 1961. Based on their genetic features, the Lake Biwa endemics were divided into three groups, the *S.* (*B.*) *decipiens* group, *S.* (*B.*) *niponica* group, and *S.* (*B.*) *habei* group. The observed genotype frequencies of the Lake Biwa endemics within each group were similar to each other. We tested the observed genotype frequencies for each polymorphic locus in each local population of the Lake Biwa endemics for departure from Hardy–Weinberg equilibrium. Most of the eight polymorphic loci of the Lake Biwa populations did not deviate significantly from HWE, except for *Aat* of *S.* (*B.*) *arenicola* at the site B8, *Pgm-1* of *S.* (*B.*) *fluvialis* at the site B9, and *S.* (*B.*) *habei* at the site B1 (level of significance $\alpha = 0.05$). The *S.* (*B.*) *niponica* and *S.* (*B.*) *habei* groups showed similar genetic features to the non-Biwa *S.* (*S.*) *libertina* and *S.* (*S.*) *reiniana* groups, whereas the *S.* (*B.*) *decipiens* group was distinct from any other group. Our allozyme data suggest that *Semisulcospira* species in Japan are distinguished robustly into two major lineages, the *S.* (*B.*) *decipiens* group and a separate non-*decipiens* group, and that all extant Lake Biwa endemics do not form a single species flock, as proposed previously.

Key words: genetic variation, allozymes, Lake Biwa, endemic speciation, *Semisulcospira*

Lake Biwa (Shiga Prefecture, Japan) has endured for about four million years and is recognized as an ancient lake, similar to Lake Tanganyika in Africa and Lake Baikal in Russia. Many endemic species in several taxa—for example, of shrimp, fish, and molluscs—have been reported in Lake Biwa (Mori and Miura 1980), as they have in other ancient lakes. In particular, 15 endemic species of the subgenus *Biwamelania* in the genus *Semisulcospira* (Caenogastropoda: Pleuroceridae) have been investigated because they may represent a single species flock (Nishino and Watanabe 2000). However, the genetic diversity of these Lake Biwa endemics has not been studied adequately. It is important to consider the genetic relationships among the Lake Biwa endemics to understand their speciation pattern during the formation of the Lake Biwa water system, and to help elucidate the reasons why so many endemic species have been maintained in the lake. In this study, we investigated the genetic variation of Japanese *Semisulcospira* species including nine Lake Biwa endemics, using allozyme analysis.

Davis (1969) studied the taxonomy of Japanese *Semisulcospira* species and divided them into two groups: the *S. libertina* species complex and the *S. niponica* species complex, based on the number of embryos in the female brood pouch, basal cords of adult shells, and chromosomes. The *S. libertina* species complex contains *S. libertina* (Gould, 1859), *S. reiniana* (Brot, 1877), and *S. kurodai* Kajiyama and Habe, 1961, which live in a widely dispersed area outside Lake Biwa. The *S. niponica* species complex consists of six Lake

* This is a posthumous paper revised by the second author.

† Present address: 3-20-5 Sumiyoshidai-higashi, Izumi-ku, Sendai 981-3222, Japan

Biwa endemic species, *S. niponica* (Smith, 1876), *S. decipiens* (Westerlund, 1883), *S. multigranosa* (Böttger, 1886), *S. reticulata* Kajiyama and Habe, 1961, *S. nakasekoae* Kuroda, 1929, and *S. habei* Davis, 1969. Habe (1978) classified the "*S. niponica* species complex" under the genus *Biwamelania*, and Matsuoka and Nakamura (1981) assigned it to the subgenus *Biwamelania* with *S. niponica* as a holotype. Watanabe (1984) described *S. morii*, which lives only around small islands in Lake Biwa. Watanabe and Nishino (1995) investigated morphological variation in the endemic species and proposed eight new species: *S. fuscata*, *S. dilatata*, *S. rugosa*, *S. ourense*, *S. takeshimensis*, *S. shiraishiensis*, *S. fluvialis*, and *S. arenicola*, classified under the subgenus *Biwamelania*. Moreover, they reported that *S. niponica* contains three morphological types, the *biwae*, nodose, and ribbed types, based on their shell sculptures. Consequently, it was suggested that many more species than previously thought are endemic to Lake Biwa.

As taxonomical research has improved, variation in shell morphology of *Semisulcospira* species has been clarified. Urabe (1992) studied two sympatric species, *S. libertina* and *S. reiniana*, from the viewpoint of genetic and morphological variation, and determined that they could be divided into two populations having unique homozygous genotypes at the locus *Mpi*: types *Mpi*-A and *Mpi*-B. She also suggested that it is difficult to identify the two species based only on their shell morphology and pointed out the individuals with the *Mpi*-B genotype could be assigned to *S. reiniana*. Recently, Kamiya and Shimamoto (2005) confirmed that the *Semisulcospira* populations with the *Mpi*-A genotype from Tohoku (northern area of Japan) to Kansai District (western area of Japan) correspond to *S.* (*S.*) *libertina*, and that the populations having the *Mpi*-B genotype in Kanto (eastern area of Japan) to Kansai District are *S.* (*S.*) *reiniana*.

A study of the phylogeny of *Semisulcospira* species was conducted by Oniwa and Kimura (1986). They examined the phylogenetic relationships of six species: *S.* (*S.*) *libertina*, *S.* (*S.*) *reiniana*, *S.* (*S.*) *kurodai*, *S.* (*B.*) *niponica*, *S.* (*B.*) *multigranosa*, and *S.* (*B.*) *nakasekoae*, through allozyme analysis, and noted large genetic differences between the Lake Biwa endemics, *S.* (*B.*) *niponica*, *S.* (*B.*) *multigranosa*, and *S.* (*B.*) *nakasekoae*, and the non-Biwa species, *S.* (*S.*) *libertina*, *S.* (*S.*) *reiniana*, and *S.* (*S.*) *kurodai*. However, the phylogenetic positions of these endemic species remain unsettled.

The fossil record of *Semisulcospira* has been studied by Matsuoka (1987). The fossils of *S.* (*B.*) *habei* occur from the Katata Formation (early to middle Pleistocene), and this species is assumed to be the oldest extant species of *Semisulcospira* (Matsuoka 1987). Nishino and Watanabe (2000) presumed that *S.* (*B.*) *habei* is a common ancestor of all extant Lake Biwa endemics, and that these endemic species were derived over a short period of time from *S.* (*B.*) *habei*.

We investigated the genetic variation and relationship among nine endemic species of the subgenus *Biwamelania*: *Semisulcospira* (*B.*) *niponica*, *S.* (*B.*) *decipiens*, *S.* (*B.*) *multigranosa*, *S.* (*B.*) *nakasekoae*, *S.* (*B.*) *fuscata*, *S.* (*B.*) *ourense*, *S.* (*B.*) *fluvialis*, and *S.* (*B.*) *arenicola* in Lake Biwa; and three non-Biwa species of the subgenus *Semisulcospira*, *S.* (*S.*) *libertina*, *S.* (*S.*) *reiniana*, and *S.* (*S.*) *kurodai*, which are widely dispersed outside Lake Biwa. Using allozyme analysis, we found that the endemic species of the subgenus *Biwamelania* can be discriminated into three groups, the *S.* (*B.*) *decipiens* group, *S.* (*B.*) *habei* group, and *S.* (*B.*) *niponica* group. The *S.* (*B.*) *habei* and *S.* (*B.*) *niponica* groups are closely related with the non-Biwa species *S.* (*S.*) *libertina*, *S.* (*S.*) *reiniana*, and *S.* (*S.*) *kurodai*, whereas the *S.* (*B.*) *decipiens* group is significantly different in its genetic features and phylogenetic position.

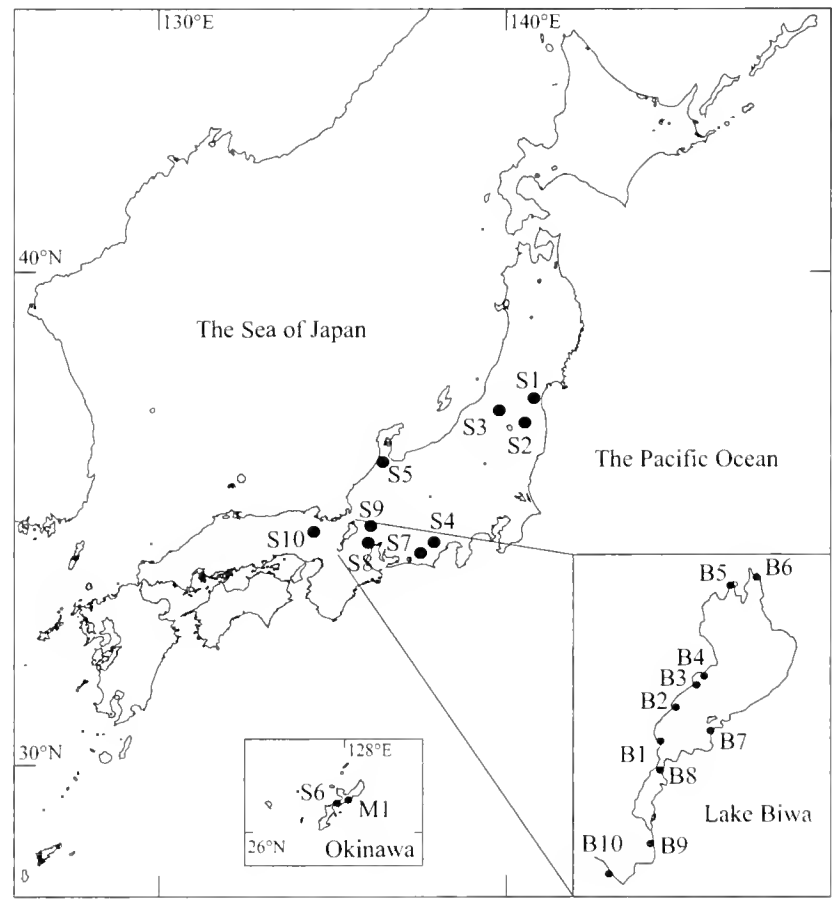

Figure 1. Map of the Japanese Islands indicating sample collection sites. S1, Sendai (Miyagi); S2, Fukushima (Fukushima); S3, Sekikawa (Niigata); S4, Fujieda (Shizuoka); S5, Kanazawa (Ishikawa); S6, On-na (Okinawa); S7, Hamamatsu (Shizuoka); S8, Komaki (Aichi); S9, Takatomi (Gifu); S10, Yoka (Hyogo); B1, Wanihama (Lake Biwa); B2, Omimaiko (Lake Biwa); B3, Shirahigehama (Lake Biwa); B4, Haginohama (Lake Biwa); B5, Oura (Lake Biwa); B6, Hannoura (Lake Biwa); B7, Mizugahama (Lake Biwa); B8, Imahama (Lake Biwa); B9, Nango (Seta River); B10, Uji (Uji River); M1, Nago (Okinawa).

Table 1. Sample collection sites and sample size of local populations, with voucher material number of the IGPS of studied specimens.

Site	Species	Locality	*N*	Inventory number
B8	*Semisulcospira* (*Biwamelania*) *arenicola* Watanabe and Nishino, 1995	Imahama, Lake Biwa	50	IGPS No. 110201
B2	*S.* (*B.*) *decipiens* (Westerlund, 1883)	Omimaiko, Lake Biwa	27	IGPS No. 110202
B3	*S.* (*B.*) *decipiens* (Westerlund, 1883)	Shirahigehama, Lake Biwa	45	IGPS No. 110203
B4	*S.* (*B.*) *decipiens* (Westerlund, 1883)	Haginohama, Lake Biwa	15	IGPS No. 110204
B9	*S.* (*B.*) *fluvialis* Watanabe and Nishino, 1995	Nango, Seta River	21	IGPS No. 110205
B5	*S.* (*B.*) *fuscata* Watanabe and Nishino, 1995	Oura, Lake Biwa	52	IGPS No. 110206
B1	*S.* (*B.*) *habei* Davis, 1969	Wanihama, Lake Biwa	16	IGPS No. 110207
B2	*S.* (*B.*) *habei* Davis, 1969	Omimaiko, Lake Biwa	18	IGPS No. 110208
B4	*S.* (*B.*) *multigranosa* (Böttger, 1886)	Haginohama, Lake Biwa	35	IGPS No. 110209
B10	*S.* (*B.*) *nakasekoae* Kuroda, 1929	Uji, Uji River	48	IGPS No. 110210
B7	*S.* (*B.*) *niponica* (nodose type) (Smith, 1876)	Mizugahama, Lake Biwa	50	IGPS No. 110211
B6	*S.* (*B.*) *niponica* (ribbed type) (Smith, 1876)	Hannoura, Lake Biwa	50	IGPS No. 110212
B5	*S.* (*B.*) *ourense* Watanabe and Nishino, 1995	Oura, Lake Biwa	52	IGPS No. 110213
S1	*Semisulcospira* (*Semisulcospira*) *libertina* (Gould, 1859)	Sendai, Miyagi	56	IGPS No. 110214
S2	*S.* (*S.*) *libertina* (Gould, 1859)	Fukushima, Fukushima	52	IGPS No. 110215
S3	*S.* (*S.*) *libertina* (Gould, 1859)	Sekikawa, Niigata	46	IGPS No. 110216
S4	*S.* (*S.*) *libertina* (Gould, 1859)	Fujieda, Shizuoka	52	IGPS No. 110217
S5	*S.* (*S.*) *libertina* (Gould, 1859)	Kanazawa, Ishikawa	52	IGPS No. 110218
S6	*S.* (*S.*) *libertina* (Gould, 1859)	On-na, Okinawa	32	IGPS No. 110219
S7	*S.* (*S.*) *reiniana* (Brot, 1876)	Hamamatsu, Shizuoka	39	IGPS No. 110220
S8	*S.* (*S.*) *reiniana* (Brot, 1876)	Komaki, Aichi	40	IGPS No. 110221
S9	*S.* (*S.*) *reiniana* (Brot, 1876)	Takatomi, Gifu	52	IGPS No. 110222
S10	*S.* (*S.*) *kurodai* Kajiyama and Habe, 1961	Yoka, Hyogo	60	IGPS No. 110223
M1	*Melanoides tuberculata* (Müller, 1774)	Nago, Okinawa	32	IGPS No. 110224

MATERIALS AND METHODS

Samples

Samples (*Semisulcospira* spp.) were collected between July 1999 and November 2002 from 21 sites on the main island of Japan (Honshu), including Lake Biwa and Okinawa Island (Fig. 1, Table 1). Thirty or more individuals were collected manually at each site within an area of approximately 10 m^2 on the bottom of a river or lake at a depth shallower than 1 m. The species *Melanoides tuberculata* (Müller, 1774) was collected in Nago, Okinawa Island (M1 in Fig. 1 and Table 1), and considered to be an outgroup of the genus *Semisulcospira* in the phylogenetic analysis.

Starch gel electrophoresis and gel staining

Starch gel electrophoresis and staining for allozyme analysis were performed as described by Fidhiany *et al.* (1988) and Shaw and Prasad (1970). Specimens were stored at –80 °C until dissection, and extracts from the digestive gland and foot were subjected to starch gel electrophoresis. Before gel electrophoresis, some pieces of digestive gland and foot were dissected, steeped in 0.5 M saccharose solution, and stored overnight at –20 °C. For gel electrophoresis, the samples were defrosted and centrifuged at 12,000 rpm for 10 min at 1°C. The supernatant was absorbed on a small strip of filter paper (4 mm × 10 mm) and then inserted in the starch gel. Gel electrophoresis was performed under constant voltage (300 V) for five or more hours with a Tris-citrate (pH 7.0) buffer

Table 2. Enzymes examined and symbols for the gene loci encoding them. E.C., Enzyme Commission numbers.

Enzyme	E. C.	Locus
Malate Dehydrogenase (MDH)	1.1.1.37	*Mdh+*, *Mdh-*
Glucosephosphate Isomerase (GPI)	5.3.1.9	*Gpi*
Isocitrate Dehydrogenase (IDH)	1.1.1.42	*Idh*
Mannose-6-Phosphate Isomerase (MPI)	5.3.1.8	*Mpi*
Phosphoglucomutase (PGM)	2.7.5.1	*Pgm-1*, *Pgm-2*
6-Phosphogluconate Dehydrogenase (6PGD)	1.1.1.44	*6pgd*
Aspartate Aminotransferase (AAT)	2.6.1.1	*Aat*
Superoxide Dismutase (SOD)	1.15.1.1	*Sod*

system (Shaw and Prasad 1970). The procedure for gel staining followed Shaw and Prasad (1970). Ten enzyme loci were resolved from eight enzymes in this study (Table 2).

Data analysis

Genotype and allelic frequencies for the 10 loci were determined for all local populations. Genotype frequencies at polymorphic loci were examined for agreement with the expectations of Hardy-Weinberg equilibrium (HWE), using a Chi-square test. The genetic relationships among 24 populations were compared using Nei's genetic distance (*D*; Nei 1972). The distance was used to cluster populations using the unweighted pair-group method with arithmetic means (UPGMA) algorithm (Sokal and Michener 1958), neighbor-joining method (Saitou and Nei 1987), and maximum likelihood (Felsenstein 1981). To investigate the robustness of nodes, we applied the bootstrap method with 1000 replicates (Felsenstein 1985). Calculation of genetic distances and construction of phylogenetic trees were performed using the Phylogeny Inference Package (PHYLIP) version 3.6 (Felsenstein 2005) and trees were drawn using TREEVIEW (Page 1996).

Embryo and adult shell treatment

After dissection, embryos and adult shells were deposited in the Museum of Natural History, Tohoku University, with a reference number given for morphological identification and for voucher specimens (Table 1). The embryos and adult shells were soaked in sodium hypochlorite solution for several minutes to remove the remaining soft body tissue, washed in distilled water, and dried at room temperature. These shells were identified morphologically following the description of Davis (1969) and Watanabe and Nishino (1995).

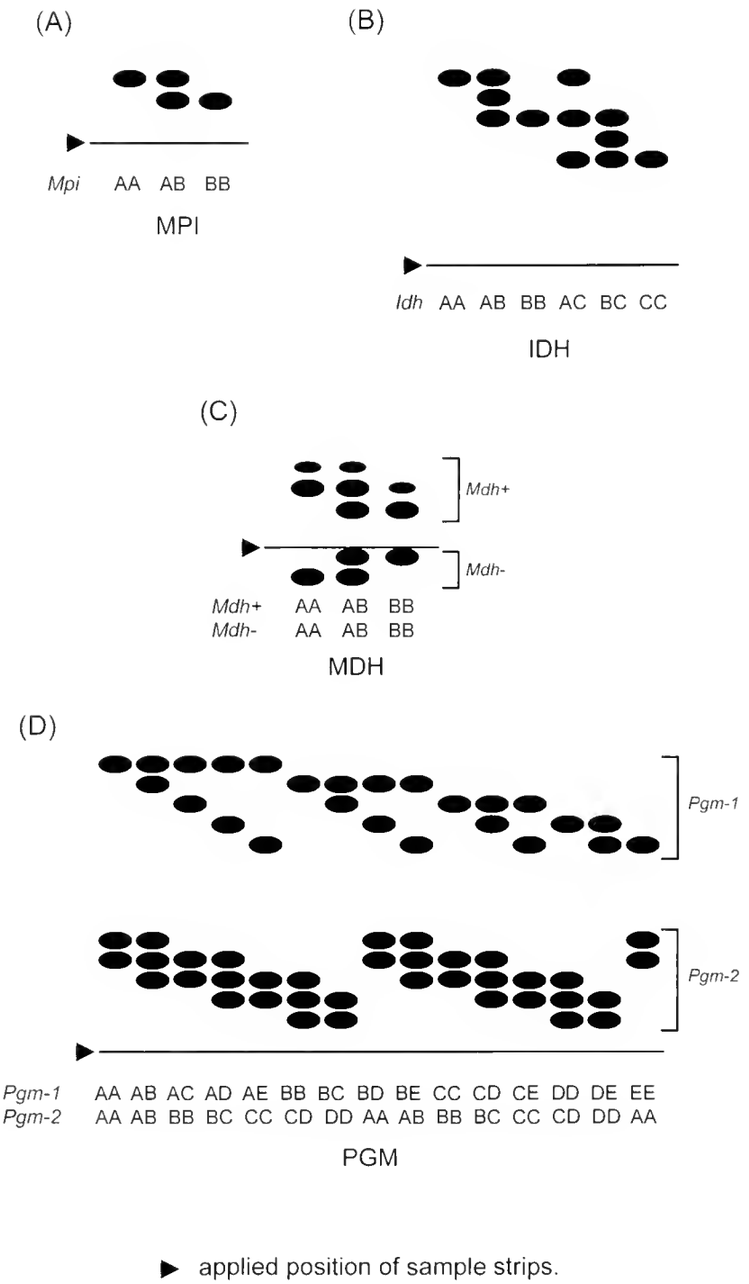

Figure 2. Schematic electrophoretic patterns of polymorphic enzymes. A, Mannose-6-phosphate isomerase (MPI); B, Isocitrate Dehydrogenase (IDH); C, Malate Dehydrogenase (MDH); D, Phosphoglucomutase (PGM).

RESULTS

Characteristics of loci

Ten gene loci for eight enzymes were detected. One or two bands were observed on the anodal side of the starch gel for the enzymes MPI and SOD. These band patterns are considered to be monomeric and controlled by a single locus, *Mpi* and *Sod*, respectively. A schema of the electrophoretic band patterns for MPI is shown (Fig. 2A). In the case of GPI, IDH, 6PGD, and AAT, one or three bands were observed on the anodal side, and the schematic band patterns of IDH are presented (Fig. 2B). These enzymes are considered to be dimeric. The enzyme MDH displayed two different band zones on the anodal and cathodal sides of the gel, and is considered to be controlled by two loci, *Mdh+* and *Mdh–*. The schematic band patterns of MDH are depicted (Fig. 2C). In *Mdh+*, one or two bands were observed on the anodal side, accompanied occasionally by a pale additional "ghost" band. Therefore, *Mdh+* was presumed to be monomeric. *Mdh–* exhibited a one- or two-banded phenotype on the cathodal side of the gel and is also monomeric. In the case of PGM, two separate migrating zones were observed on the anodal side of the gel. The schematic band patterns of PGM are shown (Fig. 2D). These two migrating zones are controlled by two loci, *Pgm-1* and *Pgm-2*, respectively. The locus *Pgm-1* displayed one- or two-banded patterns and is considered to be monomeric. However, the locus *Pgm-2* showed two- or, rarely,

three-banded patterns. Because the two-banded pattern may be interpreted as a homozygote and the three-banded pattern as a heterozygote, the locus *Pgm-2* is presumed to be monomeric.

Genetic features of each species

Based on the phenotypes of each locus, the genotype of each individual was interpreted and the allelic frequencies of each population were calculated (Table 3).

The allelic frequencies of six Lake Biwa endemics, *Semisulcospira* (*Biwamelania*) *arenicola*, *S.* (*B.*) *decipiens*, *S.* (*B.*) *fluvialis*, *S.* (*B.*) *multigranosa*, *S.* (*B.*) *nakasekoae*, and *S.* (*B.*) *ourense*, were similar to each other. At eight loci (*Mdh+*, *Mdh–*, *Gpi*, *Idh*, *Mpi*, *6Pgd*, *Pgm-2*, and *Sod*), the most frequent allele was common among all populations of these species. Especially at loci *Gpi*, *6Pgd*, and *Pgm-2*, alleles C, D, and C were predominant, respectively, and these alleles were virtually fixed in those six species. The allelic frequencies at the remaining two loci (*Pgm-1* and *Aat*) were somewhat diversified. Allele B was frequent at *Pgm-1*, and allele C was also common in all populations of these species. At the locus *Aat*, allele B was predominant, and allele A was not uncommon.

Semisulcospira (*Biwamelania*) *niponica* (ribbed type), *S.* (*B.*) *niponica* (nodose type), and *S.* (*B.*) *fuscata* are also indigenous to Lake Biwa. Alleles *Mdh–*, *Idh*, *Mpi*, and *Sod* in these species were almost fixed, as in the other six Lake Biwa endemics mentioned above, but the most fixed alleles at loci *Gpi*, *6Pgd*, and *Pgm-2*—alleles B, B and A—are quite different from those of the six Lake Biwa endemics. At loci *Aat* and *Pgm-1*, the alleles were almost fixed to B and B, respectively. At the locus *Mdh+*, allele B was predominant in the populations of *S.* (*B.*) *niponica* (nodose type) and *S.* (*B.*) *fuscata*, but allele A was more abundant in the population of *S.* (*B.*) *niponica* (ribbed type).

Semisulcospira (*Biwamelania*) *habei*, another Lake Biwa endemic, had genetic features similar to *S.* (*S.*) *kurodai*, which is found around Kinki district (in the central area of Japan) outside Lake Biwa. The alleles at the seven loci *Mdh–*, *Gpi*, *Mpi*, *6Pgd*, *Pgm-2*, *Aat*, and *Sod* were commonly fixed in the populations of the two species, as in the Lake Biwa species *S.* (*B.*) *niponica* (ribbed type), *S.* (*B.*) *niponica* (nodose type), and *S.* (*B.*) *fuscata* mentioned above. However, allele A in the locus *Idh* was frequently found in the populations of *S.* (*B.*) *habei* and *S.* (*S.*) *kurodai*, and characteristically observed in these two species. Allele B was abundant at the locus *Mdh+* of these two species, but allele A also existed in the populations of *S.* (*B.*) *habei*. The analysis of locus *Pgm-1* was complicated. Although allele B at this locus was fixed in the population of *S.* (*S.*) *kurodai*, alleles B and D coexisted in the populations of *S.* (*B.*) *habei*. Allele B was predominant in population B1 of *S.* (*B.*) *habei*, and allele D was abundant in population B2 of the species.

The allelic frequencies of the species *Semisulcospira* (*S.*) *libertina*, which inhabits river systems throughout the Japanese Islands, were highly variable among populations, except for the loci *Mdh+*, *Mdh–*, *Mpi*, *Aat*, and *Sod*. Allele B at the locus *Gpi* was abundant in the populations, except for population S4 of this species; allele C was also common in population S4. Allele B was frequent at locus *Idh*, but alleles C and A also occurred at low frequency in population S3 and S5, respectively. The allelic frequencies of loci *6Pgd*, *Pgm-1*, and *Pgm-2* were rather variable, and the most frequent allele differed by population of the species.

The genetic characterization of the species *Semisulcospira* (*S.*) *reiniana* was rather simple, except for loci *6Pgd* and *Pgm-1*. Alleles A, B, C, or D were recognized in *6Pgd*; C was the most abundant allele. The locus *Pgm-1* was almost fixed to allele B in populations S7 and S8 of this species, but alleles C and D were also common in populations S8 and S9. Allele B at locus *Mpi* was specific to populations of *S.* (*S.*) *reiniana*, whereas allele A was almost fixed in the other Japanese species examined in this study.

Genetic relationship among species and HWE test

Six Lake Biwa endemics, *Semisulcospira* (*Biwamelania*) *arenicola*, *S.* (*B.*) *decipiens*, *S.* (*B.*) *fluvialis*, *S.* (*B.*) *multigranosa*, *S.* (*B.*) *nakasekoae*, and *S.* (*B.*) *ourense*, formed a definite cluster with small *D* values ranging from 0.0009 to 0.0186 in all trees (Figs. 3-5, Table 4). These species were well discriminated from other species and their monophyly was indicated by high bootstrap values in all trees (Figs. 3-5).

Other Lake Biwa endemics, *Semisulcospira* (*Biwamelania*) *niponica* (ribbed type), *S.* (*B.*) *niponica* (nodose type), and *S.* (*B.*) *fuscata* were in the same clade with low bootstrap support in the UPGMA and maximum likelihood trees (Figs. 3, 5), but they were not in the distinct clade in the neighbor-joining tree (Fig. 4). *S.* (*B.*) *niponica* and *S.* (*B.*) *fuscata* were seen in the paraphyletic group to the *S.* (*B.*) *habei* group with low bootstrap support in the neighbor-joining tree (Fig. 4). *S.* (*B.*) *habei*, another Lake Biwa species, clustered with the non-Biwa species *S.* (*S.*) *kurodai* with *D* values ranging from 0.0053 to 0.0399 and with a high bootstrap support in all trees (Figs. 3-5).

Most of the eight polymorphic loci of the Lake Biwa populations did not deviate significantly from HWE, except for *Aat* of *Semisulcospira* (*Biwamelania*) *arenicola* (B8) and *Pgm-1* of *S.* (*B.*) *fluvialis* (B9) and *S.* (*B.*) *habei* (B1) (level of significance $\alpha = 0.05$). The observed genotype frequencies among the Lake Biwa populations were similar to each other within each above-mentioned cluster. There were no significant differences of the observed genotype frequencies even

Table 3. Allelic frequencies at 10 loci in 12 *Semisulcospira* species and outgroup. See Fig. 1 for the sample collection sites. A_0, A, B, C, D, and E indicate alleles at each locus.

	Species	*S. (B.) arenicola*	*S. (B.) decipiens*			*S. (B.) fluvialis*	*S. (B.) multigranosa*	*S. (B.) nakasekoae*	*S. (B.) ourense*	*S. (B.) fuscata*	*S. (B.) niponica* (ribbed type)	*S. (B.) niponica* (nodose type)
	Site	B8	B2	B3	B4	B9	B4	B10	B5	B5	B6	B7
Loci	*Mdh+*	A_0	-	-	-	-	-	-	-	-	-	-	-
		A	-	-	0.0111	-	-	-	-	0.0096	0.0481	0.5800	0.1064
		B	1.0000	1.0000	0.9889	1.0000	1.0000	1.0000	1.0000	0.9904	0.9519	0.4200	0.8936
	Mdh-	A	-	-	-	-	-	-	-	-	-	-	-
		B	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	Gpi	A_0	-	-	-	-	-	-	-	-	-	-	-
		A	-	-	-	-	-	-	-	-	-	0.0300	-
		B	-	-	0.0333	-	-	-	-	-	1.0000	0.9700	1.0000
		C	1.0000	1.0000	0.9667	1.0000	1.0000	1.0000	1.0000	1.0000	-	-	-
	Idh	A	-	-	-	-	-	0.0147	-	0.0294	0.0096	0.0204	0.0510
		B	1.0000	1.0000	1.0000	1.0000	1.0000	0.9853	1.0000	0.9706	0.9904	0.9796	0.9490
		C	-	-	-	-	-	-	-	-	-	-	-
	Mpi	A_0	-	-	-	-	-	-	-	-	-	-	-
		A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9896	1.0000	1.0000	1.0000	1.0000
		B	-	-	-	-	-	-	0.0104	-	-	-	-
	6Pgd	A_0	-	-	-	-	-	-	-	-	-	-	-
		A	-	-	-	-	-	-	-	-	-	-	-
		B	-	-	-	-	-	-	-	-	1.0000	0.9600	1.0000
		C	-	0.0556	0.0222	0.1071	-	-	-	0.0673	-	0.0400	-
		D	1.0000	0.9444	0.9778	0.8929	1.0000	1.0000	1.0000	0.9327	-	-	-
	Pgm-1	A_0	-	-	-	-	-	-	-	-	-	-	-
		A	-	0.0185	-	0.0333	-	-	0.0208	0.0769	0.0192	0.0100	-
		B	0.8100	0.5741	0.5889	0.7334	0.7941	0.8000	0.7396	0.8750	0.9519	0.9900	1.0000
		C	0.1800	0.3704	0.4000	0.2000	0.1177	0.1286	0.2396	0.0385	-	-	-
		D	-	-	-	-	-	-	-	-	0.0289	-	-
		E	0.0100	0.0370	0.0111	0.0333	0.0882	0.0714	-	0.0096	-	-	-
	Pgm-2	A	-	-	-	-	-	-	-	-	1.0000	1.0000	1.0000
		B	-	-	-	-	-	-	-	-	-	-	-
		C	1.0000	1.0000	1.0000	1.0000	0.9000	1.0000	0.8229	1.0000	-	-	-
		D	-	-	-	-	0.1000	-	0.1771	-	-	-	-
	Aat	A	0.2347	0.2593	0.2000	0.3333	0.2647	0.4412	0.1136	0.2981	-	-	-
		B	0.7653	0.7407	0.8000	0.6667	0.7353	0.5588	0.8864	0.7019	1.0000	1.0000	1.0000
	Sod	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3. (Continued)

		Species	*S. (B.) habei*		*S. (S.) kurodai*	*S. (S.) libertina*					*S. (S.) reiniana*			*M. tuberculata*
		Site	B1	B2	S10	S1	S2	S3	S4	S5	S6	S7	S8	S9	M1
Loci	*Mdh+*	A_0	-	-	-	-	-	-	-	-	-	-	-	-	1.0000
		A	0.1875	0.2222	-	0.0179	-	-	-	-	-	-	-	-	-
		B	0.8125	0.7778	1.0000	0.9821	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-
	Mdh-	A	-	-	-	0.0268	-	-	-	0.0096	-	-	-	-	-
		B	1.0000	1.0000	1.0000	0.9732	1.0000	1.0000	1.0000	0.9904	1.0000	1.0000	1.0000	1.0000	1.0000
	Gpi	A_0	-	-	-	-	-	-	-	-	-	-	-	-	1.0000
		A	-	-	-	-	-	-	-	-	-	-	-	-	-
		B	1.0000	1.0000	1.0000	1.0000	1.0000	0.9891	0.5481	1.0000	1.0000	1.0000	1.0000	1.0000	-
		C	-	-	-	-	-	0.0109	0.4519	-	-	-	-	-	-
	Idh	A	0.9063	0.8529	0.9000	-	-	-	-	0.0962	-	-	-	-	-
		B	0.0937	0.1471	0.1000	1.0000	1.0000	0.8478	1.0000	0.9038	1.0000	1.0000	1.0000	1.0000	1.0000
		C	-	-	-	-	-	0.1522	-	-	-	-	-	-	-
	Mpi	A_0	-	-	-	-	-	-	-	-	-	-	-	-	1.0000
		A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	-	-	-
		B	-	-	-	-	-	-	-	-	-	1.0000	1.0000	1.0000	-
	6Pgd	A_0	-	-	0.0333	-	-	-	-	-	-	-	-	-	-
		A	-	-	0.0333	-	0.1635	0.0217	-	0.0096	-	0.0880	0.1750	-	-
		B	0.9375	1.0000	0.9334	1.0000	0.2596	0.8696	-	0.0481	-	0.3870	0.3500	0.3462	-
		C	0.0625	-	-	-	0.5769	0.0978	1.0000	0.9423	1.0000	0.4250	0.4000	0.4615	1.0000
		D	-	-	-	-	-	0.0109	-	-	-	0.1000	0.0750	0.1923	-
	Pgm-1	A_0	-	-	-	-	0.1346	-	-	-	-	-	-	-	1.0000
		A	-	-	-	-	0.2500	-	-	-	1.0000	-	-	-	-
		B	0.6563	0.4722	1.0000	0.8839	0.3654	0.9783	1.0000	0.9615	-	1.0000	0.9380	0.6923	-
		C	-	-	-	0.1071	0.2500	0.0217	-	0.0289	-	-	0.0120	0.0289	-
		D	0.3437	0.5278	-	0.0090	-	-	-	0.0096	-	-	0.0500	0.2788	-
		E	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pgm-2	A	1.0000	1.0000	1.0000	-	-	0.1957	-	1.0000	1.0000	1.0000	1.0000	1.0000	-
		B	-	-	-	1.0000	0.9904	0.7935	1.0000	-	-	-	-	-	-
		C	-	-	-	-	0.0096	0.0108	-	-	-	-	-	-	1.0000
		D	-	-	-	-	-	-	-	-	-	-	-	-	-
	Aat	A	-	-	-	-	-	-	-	-	-	-	-	-	-
		B	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	Sod	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

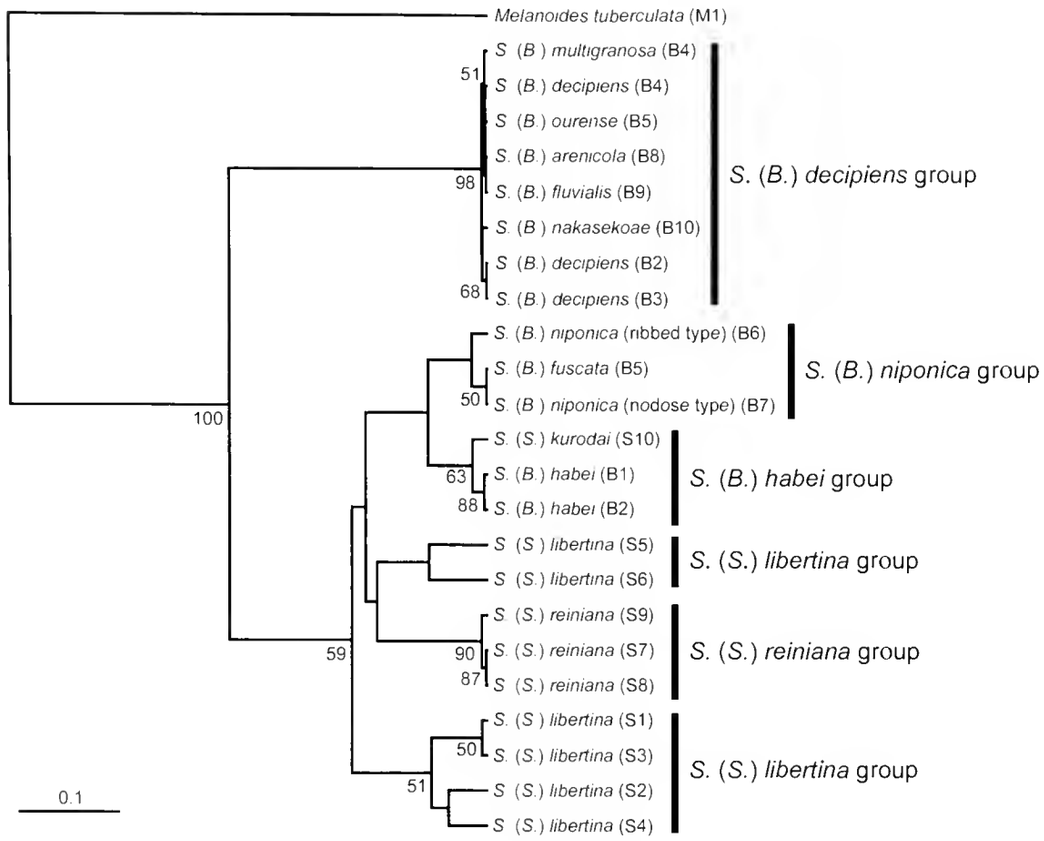

Figure 3. Relationships of *Semisulcospira* populations studied as shown in a UPGMA dendrogram. Numbers on the branches are the percentage of 1000 bootstrap replicates (≥50%). See Fig. 1 for the sample collection sites in the parenthesis.

between *S. (B.) multigranosa* and *S. (B.) decipiens* populations inhabiting the same site, B4.

The populations of *Semisulcospira (S.) libertina* did not form a distinct cluster in all trees, with relatively large *D* values ranging from 0.0097 to 0.3963 (Figs. 3- 5). Populations S5 and S6 were divergent from the cluster of populations S1, S2, S3, and S4 of *S. (S.) libertina*. The cluster of populations S5 and S6 was rather closely related to the cluster of *S. (S.) reiniana* without a significant support value in UPGMA and the maximum likelihood trees (Figs. 3, 5). Populations S7, S8, and S9 of *S. (S.) reiniana* clustered together with high bootstrap values in all trees, and their *D* values were small, ranging from 0.0010 to 0.0116 (Figs. 3- 5).

DISCUSSION

Based on the genetic features and the topologies of all of the phylogenetic trees, the 23 populations examined in the present study were clustered robustly into two major groups, the *Semisulcospira (Biwamelania) decipiens* group and non-*decipiens* group (Figs. 3-5). The non-*decipiens* group could be separated into four subclusters, the *S. (B.) niponica*, *S. (B.) habei*, *S. (S.) libertina*, and *S. (S.) reiniana* groups, although bootstrap support values among populations of these groups were not necessarily high.

The *Semisulcospira (Biwamelania) decipiens* group consists of populations of *S. (B.) arenicola*, *S. (B.) decipiens*, *S. (B.) fluvialis*, *S. (B.) multigranosa*, *S. (B.) nakasekoae*, and *S. (B.) ourense*, which are endemic species of Lake Biwa, with extremely high bootstrap support in all phylogenetic trees (Figs. 3-5). The most abundant alleles in this group are nearly fixed at loci *Gpi*, *6Pgd*, and *Pgm-2*; these loci can be used to discriminate the group from other groups (Table 3). The *D* values of each pair between species of the *S. (B.) decipiens* group and those of other groups are significantly large, averaging 0.4439 or greater (Table 5). In contrast, the *D* values of each pair of populations within the *S. (B.) decipiens* group are conspicuously small, averaging 0.0074 (Table 5). Thus, the genetic features of the *S. (B.) decipiens* group are distinct from those of any other group.

The *Semisulcospira (Biwamelania) habei* group is composed of populations of *S. (B.) habei* and *S. (S.) kurodai* with moderately high bootstrap support values; the former is a Lake Biwa species, and the latter a non-Biwa species. The *D*

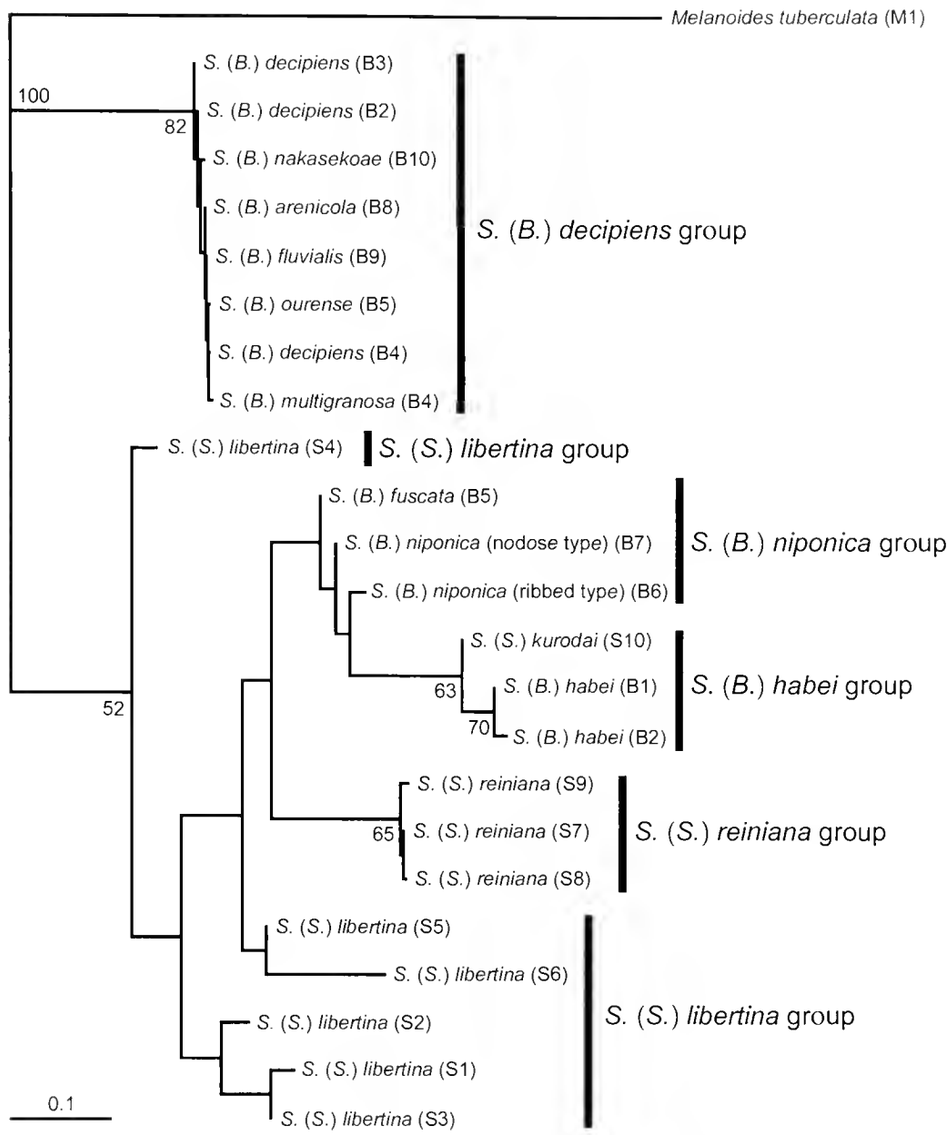

Figure 4. Relationships of *Semisulcospira* populations studied as shown in a neighbor-joining dendrogram. Numbers on the branches are the percentage of 1000 bootstrap replicates (≥50%). See Fig. 1 for the sample collection sites in the parenthesis.

values among populations of the *S.* (*B.*) *habei* group are also small, averaging 0.0211 (Table 5). The populations of this group form a single clade in all phylogenetic trees with moderately high bootstrap support (Figs. 3-5). Species of this group are closely related to those of the *S.* (*B.*) *niponica* group, with an average *D* value of 0.1192 (Table 5).

The *Semisulcospira* (*Biwamelania*) *niponica* group is composed of populations of *S.* (*B.*) *niponica* (ribbed type), *S.* (*B.*) *niponica* (nodose type), and *S.* (*B.*) *fuscata*, which are also Lake Biwa endemics, without significant support values. The *D* values among populations of the *S.* (*B.*) *niponica* group are small, with an average of 0.0204 (Table 5); these populations form a monophyletic group in UPGMA and maximum likelihood trees (Figs. 3, 5). In the neighbor-joining tree, these populations are not positioned within a distinct clade, but are closely related to the *S.* (*B.*) *habei* group (Fig. 4).

The genetic characteristics of the *Semisulcospira* (*Biwamelania*) *niponica* and *S.* (*B.*) *habei* groups are radically different from those of the *S.* (*B.*) *decipiens* group. The most abundant alleles at loci *Gpi*, *6Pgd*, and *Pgm-2* of the former two groups are entirely distinct from those of the latter *S.* (*B.*) *decipiens* group, and the mean genetic distances between the *S.* (*B.*) *niponica* or *S.* (*B.*) *habei* groups and the *S.* (*B.*) *decipiens* group are extremely large, 0.4780 or 0.6538, respectively (Table 5). In contrast, the mean genetic distances between *S.* (*B.*) *niponica* or *S.* (*B.*) *habei* groups and non-Biwa *S.* (*S.*) *libertina* or *S.* (*S.*) *reiniana* groups are rather small, 0.3097 or less (Table 5).

The populations of the *Semisulcospira* (*S.*) *reiniana* group, which are non-Biwa species, form a single cluster with moderately high bootstrap support in all phylogenetic trees (Figs. 3-5). The *S.* (*S.*) *reiniana* group is well discriminated from any other group on the basis of the allele at the locus

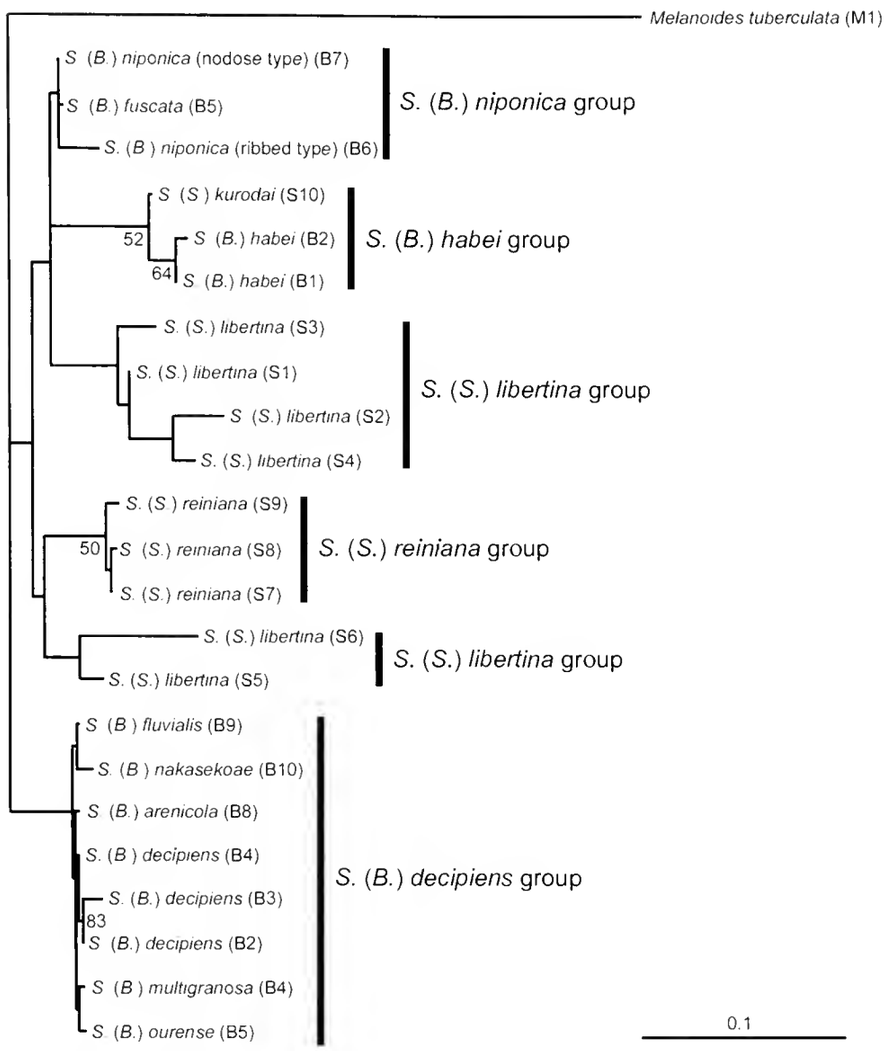

Figure 5. Relationships of *Semisulcospira* populations studied as shown in a maximum likelihood dendrogram. Numbers on the branches are the percentage of 1000 bootstrap replicates (≥50%). See Fig. 1 for the sample collection sites in the parenthesis.

MPI. Allele B at *Mpi* is fixed only in the populations of the *S.* (*S.*) *reiniana* group, whereas allele A is fixed in the populations of the other groups. The mean genetic distance among populations of the *S.* (*S.*) *reiniana* group, 0.0075, is fairly small (Table 5), although this group is distributed widely in the Kanto district and to the west in Japan.

Populations of the non-Biwa *Semisulcospira* (*S.*) *libertina* group did not form a distinct cluster with low bootstrap supports in all phylogenetic trees (Figs. 3- 5). Populations of the *S.* (*S.*) *libertina* group are distributed widely throughout Japan and exhibit the greatest genetic variability among all groups of the genus *Semisulcospira*. The mean genetic distance among populations of the *S.* (*S.*) *libertina* group, 0.1750, is remarkably large (Table 5).

As mentioned above, populations of the *Semisulcospira* (*Biwamelania*) *decipiens* group are well discriminated from any other populations examined in the present study. The *S.* (*B.*) *decipiens* group formed a definite cluster with high bootstrap support in all trees (Figs. 3-5), and, therefore, exhibit genetic features that are distinct from those of any other group. In contrast, the *D* values of each pair of populations within the *S.* (*B.*) *decipiens* group, ranging from 0.0009 to 0.0186, are conspicuously small (Table 4), and the allelic frequencies of eight populations of the *S.* (*B.*) *decipiens* group are similar to each other.

Most of the eight polymorphic loci of the 14 populations did not deviate significantly from HWE, except for *Aat* of *Semisulcospira* (*Biwamelania*) *arenicola* (B8) and *Pgm-1* of *S.* (*B.*) *fluvialis* (B9) and *S.* (*B.*) *habei* (B1) (level of significance $\alpha = 0.05$). The observed genotype frequencies of the populations of the *S.* (*B.*) *decipiens* group were very similar to each other. There were no significant differences of the observed genotype frequencies even between *S.* (*B.*) *multigranosa* and *S.* (*B.*) *decipiens* populations inhabiting the

same site B4. Based on the similar genotype frequencies and the small genetic distances, it is difficult to determine whether the eight populations of the *S.* (*B.*) *decipiens* group examined in this study are samples from a single conspecific population. However, Burch (1968) and Kobayashi (1986) have reported differences in the chromosome numbers among some Japanese *Semisulcospira* species. Burch (1968) reported that *S.* (*B.*) *multigranosa*, *S.* (*B.*) *decipiens*, *S.* (*B.*) *habei*, *S.* (*B.*) *niponica*, and *S.* (*S.*) *kurodai* have chromosome numbers of 2n = 28 to 31; 2n = 25 or 26; 2n = 17 to 20; 2n = 25 to 27; and 2n = 35 or 36, respectively. Kobayashi (1986) reported that *S.* (*B.*) *nakasekoae* has a chromosome number of 2n = 38. Moreover, remarkable interspecific differences in shell morphology have been recognized among the species of the *S.* (*B.*) *decipiens* group (Davis 1969, Watanabe and Nishino 1995). Therefore, the eight populations of the *S.* (*B.*) *decipiens* group examined in this study may not be samples from a single conspecific population; their conspicuously small *D* values may suggest that speciation has begun.

Davis (1969) studied the taxonomy of Japanese *Semisulcospira* species and divided them into two groups: the *S. libertina* species complex and *S. niponica* species complex, based on the number of embryonic shells, basal cords of adult shells, and chromosomes. The *S. libertina* species complex contained *S. libertina*, *S. reiniana*, and *S. kurodai*, which occupy a wide area around Lake Biwa and throughout Japan. The *S. niponica* species complex was composed of six Lake Biwa endemic species: *S. niponica*, *S. decipiens*, *S. multigranosa*, *S. reticulata*, *S. nakasekoae*, and *S. habei*. Habe (1978) classified the "*S. niponica* species complex" under the genus *Biwamelania*, and Matsuoka and Nakamura (1981) assigned it to the subgenus *Biwamelania* with *S. niponica* as a holotype. The species of the *S. libertina* species complex have a chromosome number of 2n = 36 (Burch 1968, Nakano *et al.* 1994), and species of the *S. niponica* species complex have fewer chromosomes, 2n = 17 to 31 (Burch 1968). The former species are characterized by having seven or more basal cords of adult shells and 100 or more embryonic shells in the female brood pouch; the latter species are characterized by having from two to six basal cords and fewer embryonic shells (Davis 1969). Although the *S.* (*S.*) *kurodai* was placed in the *S. libertina* species complex by Davis (1969), the species was considered transitional between the two species complexes, as the adult shells have an average 5.1 basal cords and about 50 embryos per female brood pouch. All phylogenetic trees in the present study (Figs. 3-5) support the arguments of Davis (1969). Our allozyme data suggest that the *S.* (*S.*) *kurodai* relates intimately to not only the *S.* (*B.*) *habei* and *S.* (*B.*) *niponica* groups, especially to *S.* (*B.*) *habei*, but also *S.* (*S.*) *libertina* and *S.* (*S.*) *reiniana*.

Matsuoka (1987) presumed *Semisulcospira* (*Biwamelania*) *habei* to be the oldest extant species in Lake Biwa. Moreover, Nishino and Watanabe (2000) considered *S.* (*B.*) *habei* to be a common ancestor of all extant Lake Biwa species, with all extant Lake Biwa species derived from *S.* (*B.*) *habei* over a short period of time. As mentioned, *S.* (*B.*) *habei* was not positioned in a basal clade in all phylogenetic trees in the present study (Figs. 3- 5), and *S.* (*B.*) *habei* was found to be related intimately to both the Lake Biwa *S.* (*B.*) *niponica* group and the non-Biwa *S.* (*S.*) *libertina* and *S.* (*S.*) *reiniana* groups. In contrast, the Lake Biwa *S.* (*B.*) *decipiens* group was found to be genetically distant from the other Lake Biwa taxa including *S.* (*B.*) *habei*. Therefore, the notion that *S.* (*B.*) *habei* is the oldest common ancestor of all extant Lake Biwa species should be reconsidered.

Recently, Lee *et al.* (2007) investigated the population genetic structure and phylogenetic relationships of four morphospecies of *Semiculcospira* sampled from multiple South Korean drainages. They reported a taxonomically heterogeneous species complex containing population-level admixtures of genotypes, and they considered that it might be attributed to a number of generative mechanisms, such as ancestral polymorphisms, hybridization, and the existence of cryptic species. In the present study, several *Semiculcospira* species did not form a robust monophyletic clade, except *S.* (*B.*) *habei* and *S.* (*S.*) *reiniana* (Figs. 3-5). As pointed out by Lee *et al.* (2007), comprehensive phylogenetic studies and research on ecophenotypic plasticity in shell morphology of the Japanese *Semiculcospira* species may be needed in future.

CONCLUSION

We investigated the genetic variations of 12 living Japanese species of the gastropod genus *Semisulcospira*, including 9 Lake Biwa endemics, based on allozyme analysis. The Lake Biwa endemics were genetically discriminated into three groups, the *S.* (*Biwamelania*) *decipiens*, *S.* (*B.*) *niponica*, and *S.* (*B.*) *habei* groups. The genetic features of six Lake Biwa species of the *S.* (*B.*) *decipiens* group are distinct from those of any other *Semisulcospira* species examined in the present study. In contrast, other Lake Biwa species of the *S.* (*B.*) *niponica* and *S.* (*B.*) *habei* groups are closely related to each other and to the non-Biwa species, *S.* (*S.*) *libertina* and *S.* (*S.*) *reiniana*. Our allozyme data suggest that the Japanese *Semisulcospira* species are distinguished robustly into two major lineages, a *S.* (*B.*) *decipiens* group and a separate non-decipiens group, and that all extant Lake Biwa endemics do not form a single species flock, as proposed previously.

Table 4. Nei's genetic distances (*D*) among *Semisulcospira* populations. See Fig. 1 for the sample collection sites in the parenthesis. Numbers (1 to 24) indicate local population.

	Population	1	2	3	4	5	6	7	8	9	10	11
1:	*S. (B.) arenicola* (B8)											
2:	*S. (B.) decipiens* (B2)	0.0059										
3:	*S. (B.) decipiens* (B3)	0.0062	0.0009									
4:	*S. (B.) decipiens* (B4)	0.0028	0.0045	0.0072								
5:	*S. (B.) fluvialis* (B9)	0.0018	0.0088	0.0099	0.0041							
6:	*S. (B.) multigranosa* (B4)	0.0055	0.0114	0.0150	0.0036	0.0051						
7:	*S. (B.) nakasekoae* (B10)	0.0061	0.0098	0.0081	0.0116	0.0052	0.0186					
8:	*S. (B.) omense* (B5)	0.0029	0.0130	0.0153	0.0034	0.0036	0.0046	0.0126				
9:	*S. (B.) fuscata* (B5)	0.4462	0.4712	0.4578	0.4543	0.4395	0.4777	0.4237	0.4424			
10:	*S. (B.) niponica* (ribbed type) (B6)	0.5163	0.5477	0.5302	0.5275	0.5106	0.5523	0.4936	0.5101	0.0337		
11:	*S. (B.) niponica* (nodose type) (B7)	0.4526	0.4804	0.4663	0.4618	0.4461	0.4844	0.4307	0.4479	0.0008	0.0269	
12:	*S. (B.) habei* (B1)	0.6611	0.6824	0.6644	0.6719	0.6558	0.6986	0.6314	0.6501	0.1152	0.1386	0.1079
13:	*S. (B.) habei* (B2)	0.6893	0.7018	0.6832	0.6993	0.6836	0.7284	0.6560	0.6829	0.1236	0.1450	0.1182
14:	*S. (S.) kurodai* (S10)	0.5989	0.6348	0.6180	0.6134	0.5937	0.6321	0.5758	0.5849	0.0959	0.1400	0.0883
15:	*S. (S.) libertina* (S1)	0.4452	0.4635	0.4498	0.4521	0.4395	0.4778	0.4207	0.4455	0.1226	0.1698	0.1250
16:	*S. (S.) libertina* (S2)	0.4472	0.4309	0.4220	0.4336	0.4424	0.4832	0.4127	0.4475	0.2266	0.2860	0.2352
17:	*S. (S.) libertina* (S3)	0.4124	0.4373	0.4244	0.4193	0.4063	0.4441	0.3904	0.4076	0.0827	0.1300	0.0839
18:	*S. (S.) libertina* (S4)	0.3339	0.3470	0.3463	0.3216	0.3265	0.3615	0.3128	0.3183	0.2946	0.3442	0.2979
19:	*S. (S.) libertina* (S5)	0.4427	0.4574	0.4499	0.4321	0.4363	0.4742	0.4200	0.4274	0.1100	0.1481	0.1111
20:	*S. (S.) libertina* (S6)	0.5971	0.5653	0.5641	0.5648	0.5884	0.6332	0.5554	0.5777	0.2454	0.2985	0.2579
21:	*S. (S.) reiniana* (S7)	0.5786	0.6104	0.5962	0.5854	0.5735	0.6178	0.5511	0.5712	0.1629	0.2110	0.1652
22:	*S. (S.) reiniana* (S8)	0.5855	0.6143	0.5999	0.5917	0.5805	0.6255	0.5567	0.5794	0.1671	0.2167	0.1702
23:	*S. (S.) reiniana* (S9)	0.5858	0.6020	0.5879	0.5895	0.5803	0.6263	0.5528	0.5847	0.1828	0.2372	0.1888
24:	*M. tuberculata* (M1)	0.8327	0.8030	0.8011	0.8068	0.8528	0.8817	0.8332	0.8288	1.0904	1.0527	1.0996

Table 5. Mean genetic distances between *Semisulcospira* species groups. Numbers (1 to 5) indicate species group.

	Species group	1	2	3	4	5
1:	*S. (B.) decipiens* group	0.0074				
2:	*S. (B.) habei* group	0.6538	0.0211			
3:	*S. (B.) niponica* group	0.4780	0.1192	0.0204		
4:	*S. (S.) libertina* group	0.4439	0.3097	0.1983	0.1750	
5:	*S. (S.) reiniana* group	0.5886	0.3115	0.1891	0.2865	0.0075

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Table 4. (Continued)

12	13	14	15	16	17	18	19	20	21	22	23	24
0.0053												
0.0180	0.0399											
0.2644	0.2735	0.2357										
0.3655	0.3749	0.3581	0.0794									
0.2058	0.2212	0.1735	0.0097	0.0823								
0.4639	0.5056	0.4203	0.1525	0.0776	0.1340							
0.2102	0.2406	0.1880	0.2495	0.1784	0.1756	0.1533						
0.3491	0.3532	0.3716	0.3963	0.1973	0.3320	0.2897	0.1170					
0.3143	0.3397	0.2760	0.3142	0.3357	0.2527	0.3441	0.1537	0.2994				
0.3156	0.3377	0.2817	0.3199	0.3320	0.2585	0.3499	0.1563	0.2945	0.0010			
0.3127	0.3206	0.3056	0.3390	0.3291	0.2804	0.3647	0.1644	0.2679	0.0116	0.0099		
1.3673	1.3682	1.4380	1.0910	0.8042	1.0613	0.7826	0.8313	0.8109	0.9285	0.9270	0.8921	

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Hand harvesting quickly depletes intertidal whelk populations

Jacob D. Shalack, Alan J. Power, and Randal L. Walker

University of Georgia, Marine Extension Service, Shellfish Research Laboratory, 20 Ocean Science Circle, Savannah, Georgia 31411-1011, U.S.A.

Correspondence, A. J. Power: alanpowr@uga.edu

Abstract: With the collapse of the offshore whelk trawl fishery in Georgia, interest has increased in harvesting whelk from inshore areas where trawling is prohibited. This study examines the effects of hand harvesting on local intertidal populations of whelk. Over a 34 day period from 27 February to 1 April 2006, 1,824 whelk were hand harvested at low tide: 91.2% knobbed, *Busycon carica* (Gmelin, 1791); 4.7% lightning, *Busycotypus sinistrum* (Hollister, 1958); 4.1% channeled, *Busycotypus canalicalatus* (Linnaeus, 1758); and one pearwhelk, *Busycotypus spiratus* (Lamarck, 1816). Significantly greater numbers of knobbed and lightning female whelk were found than males. Mean shell lengths for females were consistently larger than males of all species. The study period was divided into three collection periods that were separated by approximately one week. The numbers of whelk harvested and their mean shell lengths significantly decreased between sampling periods as stocks were depleted. All species and sexes were active in both daytime and nighttime; however, significantly more knobbed and channeled whelk were harvested when sampling occurred closer to the middle of the night and significantly fewer toward the middle of the day. Nocturnal feeding is likely a cryptic adaptation to avoid predation and desiccation, and many whelk presumably remain buried on the intertidal flats during diurnal exposure. This study was conducted in the period leading up to copulation and egg-laying on the intertidal sandy-mud flats in inshore areas in coastal Georgia. Nocturnal hand harvesting at this time of the year could very quickly have detrimental impacts to local whelk stocks. Further implications of this work for an intertidal hand harvest supplemental whelk fishery are discussed.

Key words: behavior, whelk, fishery, intertidal, nocturnal

Four species of whelk (Family Melongenidae) occur in the coastal waters of Georgia: the knobbed whelk *Busycon carica* (Gmelin, 1791), the lightning whelk *Busycon sinistrum* (Hollister, 1958), the channeled whelk *Busycotypus canalicalatus* (Linnaeus, 1758), and the pearwhelk *Busycotypus spiratus* (Lamarck, 1816) (Abbott 1974). The first three species are common, while the pearwhelk is rare (Walker 1988). Georgia's coastal water temperatures typically follow a seasonal pattern, ranging from 8-10 °C in winter to 30-32 °C in summer months. Seasonal intertidal whelk abundances have been associated with reproductive and feeding behavior (Magalhaes 1948, Walker 1988, Power *et al.* 2002a, Walker *et al.* 2004, 2008).

The Georgia Department of Natural Resources' Coastal Resources Division first authorized the commercial harvest of whelk in 1980 (Belcher *et al.* 2001). The fishery developed primarily as an offshore winter fishery when shrimpers changed gear, utilizing nets with a larger mesh and heavy chain attached to the bottom edge of the net to enhance bottom harvesting. Whelk landings peaked in 1990 in Georgia, where 462,196 kg of meat valued at $507,718 was reported (Georgia Department of Natural Resources 2010). The fishery remained viable through the 1990s, but landings began to decrease drastically in 2000. The average annual landings for the period 2004-2008 have been 1,372 kg of meat, valued at $3,832 (Georgia Department of Natural Resources 2010). The collapse of the trawl fishery led to an increase in price from $1.10 per kg in the early 1990s to $2.72 per kg in 2007, which resulted in mounting interest in harvesting whelk from the inshore areas (typically depths up to 6 m) where trawling is prohibited. Prices have since subsided back to 1990's levels and currently can reach up to $1.21 per kg.

Blue crab, *Callinectes sapidus*, fishers have been harvesting channeled whelk from inshore waters for years. Georgia crabbers typically soak crab traps baited with Atlantic Menhaden (*Brevoortia tyrannus*) overnight to catch blue crabs. However, Walker *et al.* (2003) determined a combination of fish and crab bait was more effective for directly targeting whelk. Studies have shown that local stocks can be depleted within weeks through pot fishing (Walker *et al.* 2003, Shalack 2007). Most of Georgia's hard clam *Mercenaria mercenaria* (Linné, 1758) (Walker *et al.* 1980, Walker and Rawson 1985) and oyster *Crassostrea virginica* (Gmelin, 1791) (Harris 1980) stocks occur in the intertidal zone. Shellfishermen have traditionally hand gathered knobbed whelk from these intertidal areas (Walker 1988). Commercial clam and oyster fishermen do not currently have a possession limit for hand-harvested whelk from the intertidal zone and may collect year around and at any hour of the day. Licensed recreational fishermen are permitted to take up to 1 bushel of whelk per day.

In previous studies, whelk have been reported to be abundant in the study area during the time of year that this

work was conducted. This period typically leads up to the start of copulation and egg deposition on the intertidal mud flats in early April (Walker 1988, Power *et al.* 2002a). The Georgia coast experiences a semi-diurnal tidal cycle with a tidal range up to 2.3 m. Hand picking of whelk by intertidal clam and oyster fishers would produce large yields at this time of the year, but could have negative impacts on population recruitment. In this study we hand harvested whelk from an intertidal habitat in Georgia to quantitatively describe the whelk community composition, abundance, demographics, and sex ratios with regard to tidal stage, time of day, ambient temperatures (air and water), and moon phase. This information will have value in the management of a sustainable inshore whelk fishery.

MATERIALS AND METHODS

Whelk were hand collected from the intertidal area of Dead Man Hammock, Wassaw Island, Georgia from 27 February to 1 April 2006 (Fig. 1, Appendix 1). Catch per unit effort was defined as the number of whelk captured during each collection period. Given that the same physical area was searched during each low tide, it was determined that the time taken to collect whelk each time was irrelevant. Harvesting occurred during low tides by walking along the contours of the oyster reefs (approx. 450 m) and searching a 60-m swath of the adjacent mud flat. Harvesting occurred on days that the tide receded enough to permit collecting in the lower intertidal zone. This resulted in three distinct collection periods that were separated by approximately one week over the course of the entire study period. On the first day of collection, harvesting started after the tide had turned; all other collections started with the outgoing tide.

Day collections are defined as occurring between the hours of sunrise and sunset. The time of day for each collection was further defined by dividing both the day and night into eight light intensity intervals. Four equal quarters were established from sunrise to the middle of the day and were ranked from 0 to 4, while the four subsequent quarters to sunset were ranked from 4 to 0. Likewise the quarters from sunset to the middle of the night were ranked from 0 to -4 and from -4 to 0 for the quarter to sunrise. The light intensity was determined by averaging the score of the intervals during which each collection occurred. The extent of the tidal cycle was defined by the mean lower low water (MLLW) reported by NOAA (tidesandcurrents.noaa.gov) for the study site. The lunar phase was also recorded for each night time collection by recording the percentage of the moon visible as reported by the US Naval Observatory (www.usno.navy.mil). Surface water and air temperatures were taken with a thermometer on site during collections.

Harvested whelk were returned to the laboratory, where they were held in separate raceways until they could be identified to species and measured for shell length (siphonal canal to shell apex). Each whelk was identified by super gluing an identifying Hallprint® plastic tag to the body whorl near the top of the aperture. Tagged whelk were then relaxed in seawater containing 7% magnesium chloride and sex was determined by the presence or absence of a penis. In this region, imposex is not a commonly observed phenomenon in these species. Whelk recovered completely after sexing but were not released until the study was completed to avoid recapture and confounding the impacts of hand-harvesting fishing pressure.

Size frequency distributions were plotted for male, female, and unsexed knobbed whelk for each of the three collection periods and for the entire collection period. Since fewer channeled and lightning whelk were harvested, combined size frequency distributions for all collection periods were only plotted for these species. A goodness-of-fit *G*-test was calculated for the number of males to females across these categories to identify those

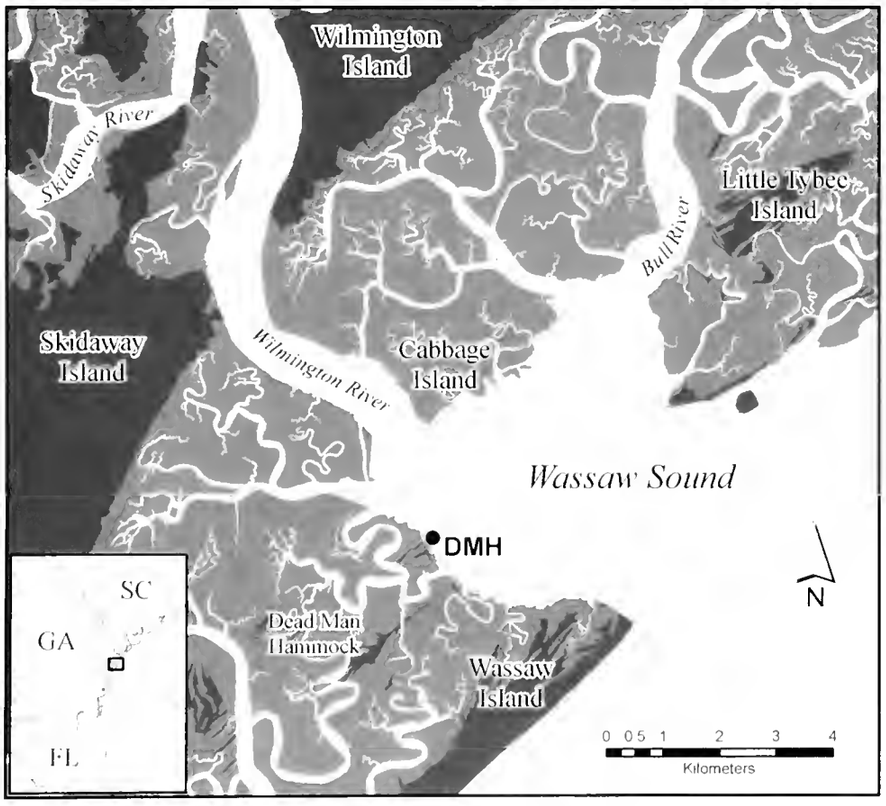

Figure 1. Dead Man Hammock study site on Wassaw Island, Georgia (31°55′11″N 80°58′19″W).

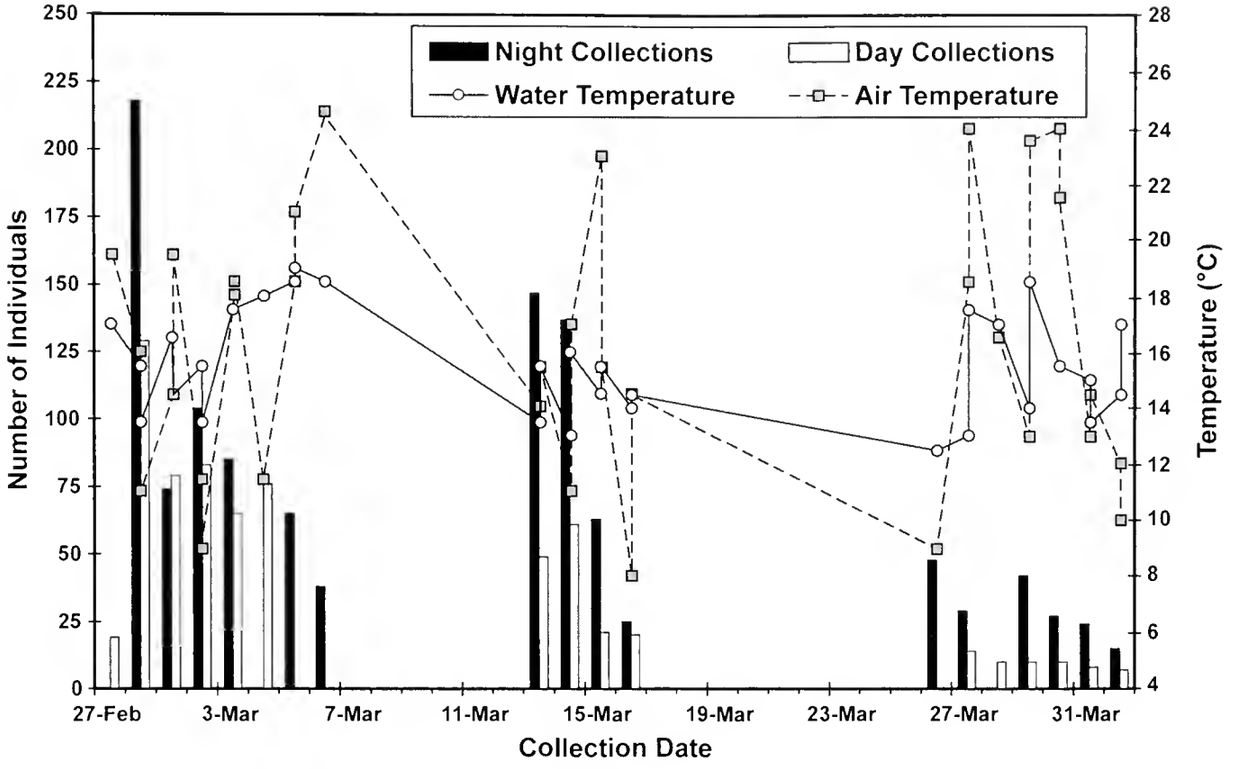

Figure 2. The number of diurnal and nocturnal whelks harvested over the three collection periods between Feb. 27 and Apr. 1 and the corresponding on-site ambient water and air temperatures (°C).

collections significantly different from a 1:1 ratio. Only one male lightning and one pear whelk were identified and, therefore, were excluded from this analysis. ANOVAs were used to test for significant differences between the numbers of each whelk species captured during daylight versus during the night (SAS Institute Inc. 2008). A factorial two-way ANOVA was used to compare the mean sizes of male and female knobbed whelk captured during daylight versus night hours (SAS Institute Inc. 2008). Spearman's rank correlation analysis was used to determine significant correlations

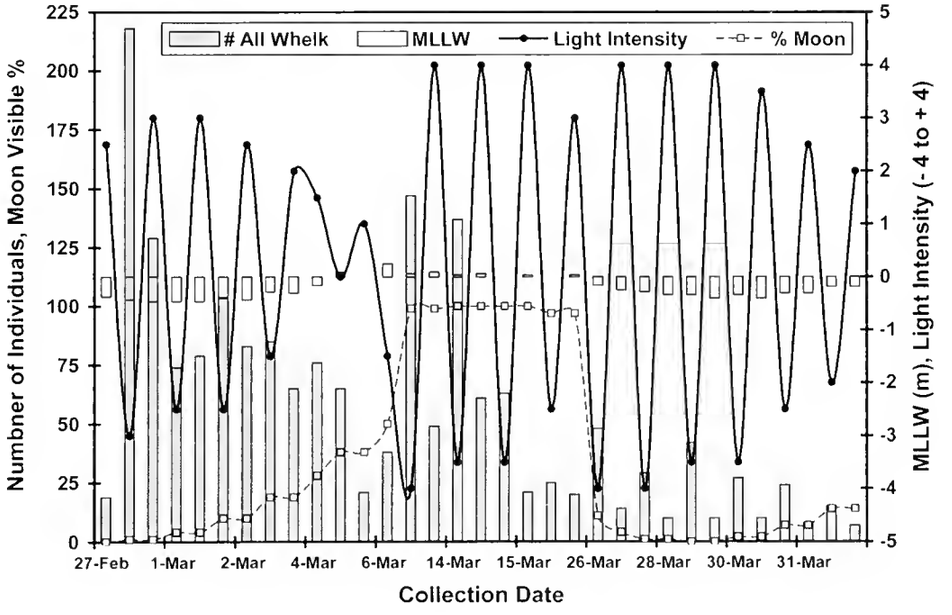

Figure 3. The number of whelk individuals collected in relation to date, tide (mean lower low water), light intensity (-4 to +4), and lunar phase (0 to 100%).

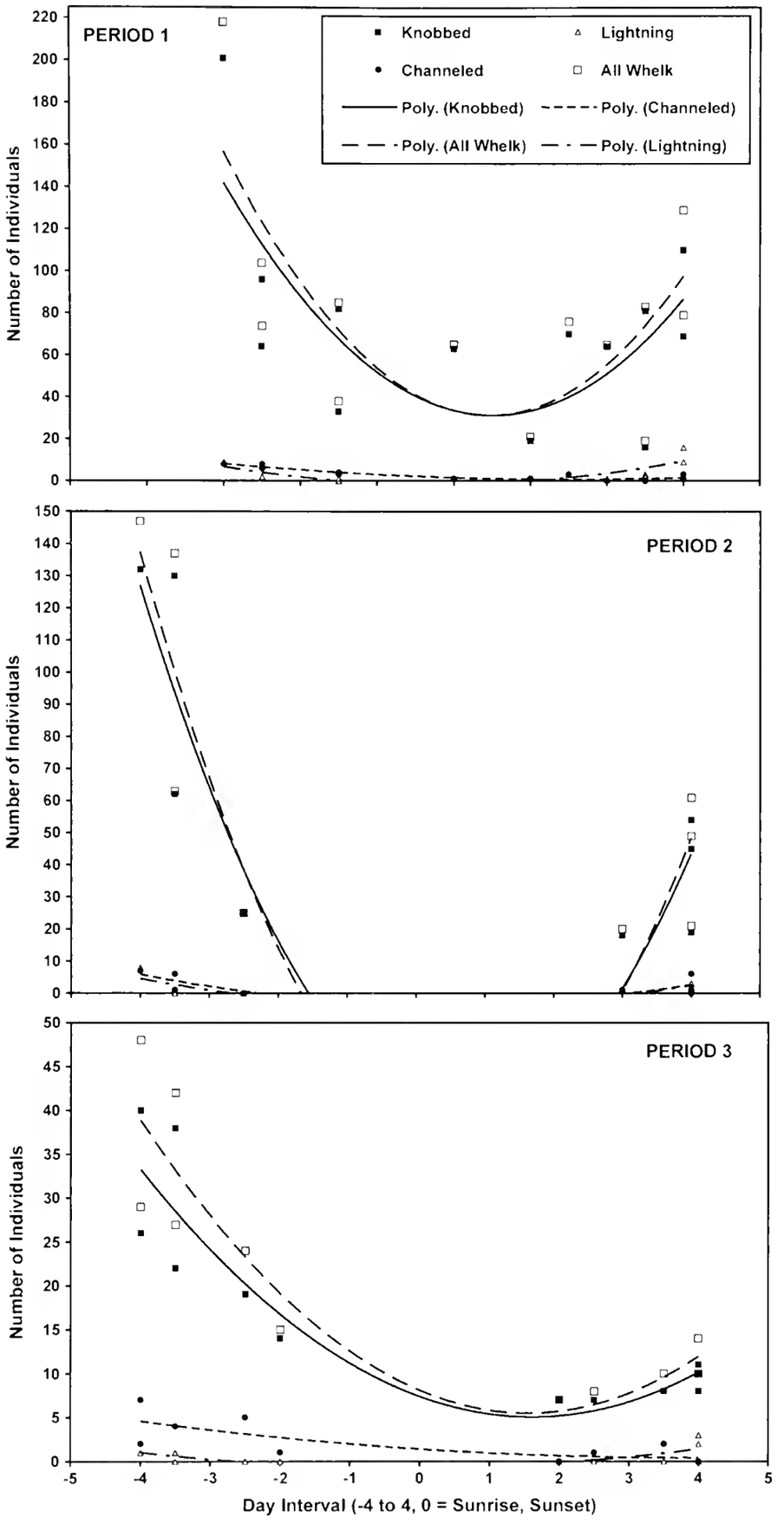

Figure 4. The numbers of individual knobbed, lightning, channeled, and all whelks harvested during the three collection period (Feb. 27 to Mar. 06, Mar. 13 to Mar. 16, and Mar. 26 to Apr. 1) in relation to the mean collection time light intensity (-4 to +4).

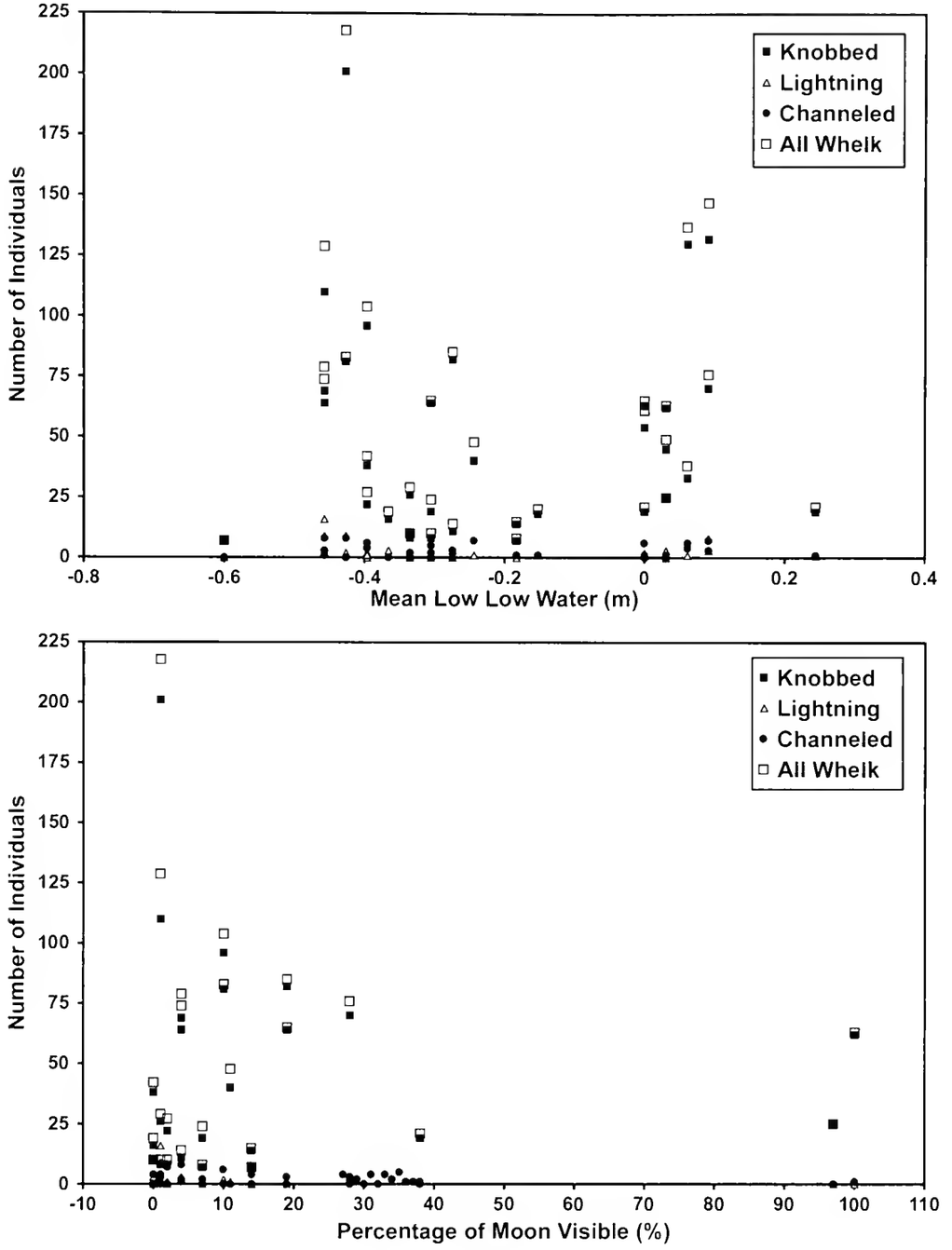

Figure 5. The numbers of individual knobbed, lightning, channeled, and all whelks harvested during the three collection periods (Feb. 27 to Apr. 1) in relation to the extent of the tidal stage as defined by mean lower low water from NOAA (upper panel) and in relation to the lunar phase as quantified by the percentage of the moon visible by the US Naval Observatory (lower panel).

between the numbers, sizes, and sexes of whelk and the corresponding tidal height, time of day, moon phase, and air and water temperatures. Polynomial and linear trend lines were fitted using Excel to further explore the relationships between the various parameters and changes in whelk species abundances over the study period. An ANOVA and an ANCOVA (using light intensity as a covariate) were also used to determine the significance of changes in the numbers of whelk captured between the three sampling periods (SAS Institute Inc. 2008).

RESULTS

Water and air temperatures were typical of coastal Georgia during the study period (Fig. 2). Water temperatures ranged from 13 to 19 °C during daylight collections and from 12.5 to 18.5 °C during night collections. Daytime aerial temperatures ranged from 11.5 °C to 24.5 °C and nighttime aerial temperatures from 9 °C to 18.5 °C. Day length increased from 11 hours 27 minutes to 12 hours and 31 minutes over the course of the study period, which consisted

Table 1. Spearman's rank correlations for (a) numbers of all whelks, (b) mean shell lengths (SL) of sexed knobbed whelks, and (c) mean shell lengths of sexed lightning and channeled whelks. Correlations are provided for: sample sizes, male to female ratios, mean shell lengths, mean lower low water (MLLW), percentage of moon visible (for collections with mean light intensities 0 to -4), water and air temperatures, and the mean light intensity interval recorded during each collection for all samples. Only one male lightning whelk was identified and is not included in (c). Significant correlations at $P < 0.05$ are indicated with an * and in boldface font.

(a)	Knobbed (#)	Channeled (#)	Lightning (#)	All whelk (#)
Mean shell length	**0.34***	-0.15	0.35	**0.56***
MLLW	-0.05	0.01	-0.17	-0.06
% Moon visible	0.23	-0.29	-0.31	0.22
Water temperature	-0.16	-0.20	**-0.40***	-0.17
Air temperature	-0.24	-0.33	0.01	-0.24
Light intensity	**-0.40***	**-0.59***	0.19	**-0.41***

(b)	All knobbed (SL)	Male knobbed (SL)	Female knobbed (SL)
Numbers	**0.34***	**0.60***	0.22
M:F ratio	**-0.63***	**-0.59***	-0.30
MLLW	-0.30	-0.31	**-0.35***
% Moon visible	-0.07	0.39	0.04
Water temperature	**-0.40***	-0.07	-0.26
Air temperature	-0.01	0.03	0.01
Light intensity	0.18	-0.03	0.24

(c)	All lightning (SL)	Female lightning (SL)	All channeled (SL)	Male channeled (SL)	Female channeled (SL)
Numbers	0.35	**-0.58***	-0.15	-0.03	-0.35
MLLW	-0.27	**-0.53***	0.30	0.25	0.14
% Moon visible	**-0.78***	-0.60	0.34	**0.73***	0.46
Water temperature	-0.22	**-0.64***	0.02	0.09	0.33
Air temperature	-0.02	-0.39	-0.14	0.15	0.25
Light intensity	-0.08	-0.45	-0.11	0.18	0.18

of three discrete sampling periods separated by approximately one week. Collections occurred throughout the range of light intensity intervals established (-4 to +4, Figs. 3-4) and during tides ranging from -46 to 24 cm (Fig. 5). Nighttime collections occurred from new (0%) to full (100%) moons, but no collections occurred between the range of 40 and 90% (Fig. 5).

A total of 1,824 whelk were gathered by hand at Dead Man Hammock on Wassaw Island, Georgia from 27 February 2006 to 1 April 2006. There was a significant difference between the number of each species harvested (ANOVA, $F = 25.56$, $P < 0.01$). Most whelk (91.2%) were knobbed (*Busycon carica*), with 4.7% being lightning (*Busycotypus sinistrum*) and 4.1% channeled (*Busycotypus canaliculatus*). Only one pearwhelk (*Busycotypus spiratus*) was found. The numbers of whelk captured at night (- light intensity) versus during the day (+ light intensity) was significant only for channeled whelk (ANOVA, $F = 14.71$, $P < 0.01$). Spearman's rank correlation coefficients did, however, determine a significant negative association between the mean light intensity interval and the number of knobbed ($R = -0.40$), channeled ($R = -0.59$), and all whelk ($R = -0.41$) at $P < 0.05$ (Table 1).

Table 2. Equations of polynomial lines ($y = ax^2 + bx + c$) fitted to Figs. 4-6 to describe the numbers of individual knobbed, lightning, channeled, and all whelks harvested during each collection period in relation to the mean collection time light intensity.

Period 1	a	b	c	R^2
Knobbed	8.99	-9.23	33.58	0.50
Channeled	0.37	-1.11	1.48	0.85
Lightning	1.06	0.45	-1.52	0.60
All	10.42	-9.89	33.53	0.53
Period 2	**a**	**b**	**c**	**R^2**
Knobbed	7.48	-10.47	-34.41	0.77
Channeled	0.48	-0.40	-3.39	0.42
Lightning	0.47	-0.26	-4.11	0.44
All	8.43	-11.14	-41.92	0.76
Period 3	**a**	**b**	**c**	**R^2**
Knobbed	0.89	-2.89	7.38	0.84
Channeled	0.07	-0.52	1.40	0.62
Lightning	0.12	0.05	-0.69	0.40
All	1.08	-3.36	8.09	0.84

The numbers of individual knobbed, lightning, channeled, and all whelk harvested during each of the three collection periods, and according to the day interval in which collection occurred is illustrated (Fig. 4). Polynomial lines and equations (Table 2) are also provided to describe the data. Fewer whelk per collection period were harvested closer to the middle of the day and *vice versa* for night collections. The abundance of lightning whelk was negatively correlated ($P < 0.05$) with water temperature ($R = -0.40$). The extent of tidal height had no significance on the abundances of any of the whelk species.

The sex ratios and size frequency distributions for male and female whelk collected during each period and for the entire collection period are summarized (Table 3) and illustrated (Fig. 6). Given the low numbers of channeled and lightning whelk, only cumulative histograms are presented for these species (Fig. 7). Knobbed whelk ranged in size from 39 to 234 mm, lightning from 82 to 229 mm, and channeled from 50 to 141 mm. Females dominated the larger size classes for all whelk. For knobbed whelk, females (mean = 126.9 mm) were significantly larger than males (mean = 87.4 mm) at $P < 0.01$. Significantly larger-sized knobbed specimens were also found at night (ANOVA, $F = 9.79$, $P < 0.01$); females collected during the day averaged 121.2 mm, females collected at night averaged 132.7 mm, males collected during the day averaged 86.4 mm, males at night averaged 88.3 mm. Shell length was significantly ($P < 0.05$) and positively correlated with sample size for all whelk ($R = 0.56$), all knobbed ($R = 0.34$), and male knobbed whelk ($R = 0.60$), but was negatively correlated with sample size of female lightning whelk (-0.58). Female knobbed and lightning whelk sizes were negatively and significantly ($P < 0.05$) correlated with MLLW ($R = -0.35$ and -0.53), indicating that lower tides resulted in harvesting larger specimens. Male channeled whelk were positively correlated ($R = 0.73$) with the percentage of the moon that was visible, but lightning whelk were negatively correlated ($R = -0.78$). Finally, the mean shell length of all knobbed and female lightning whelk was significantly and negatively correlated with water temperatures ($R = -0.40$ and -0.64, respectively).

The male to female ratio for knobbed whelk ranged from 0 to 1.33, and females dominated most samples for each species harvested. The male to female ratio for all whelk and knobbed whelk was 0.24, and for lightning whelk was 0.02 (Table 3), all of which were significantly different from a 1:1 ratio ($P < 0.01$). The male to female ratio for all channeled whelk was 0.69, which was not significantly different from parity. Given that females dominated the larger size categories it was not surprising that the male to female ratio was inversely correlated to shell length for all knobbed and male knobbed whelk ($R = -0.63$ and -0.59).

The sex ratio and size structure of the intertidal whelk population changed over the course of the three collection periods with a general trend of increasing males and smaller sizes and numbers caught as harvesting progressed (Table 3). Given the small sample sizes of channeled and lightning whelk, they were excluded from detailed analysis. There was a significant difference in the mean number of all whelk caught per sampling trip between the three sampling periods ($P < 0.001$), declining from 86.4 to 65.4, and finally 20.3. Similarly, the mean number of knobbed whelk was statistically different between periods ($P < 0.001$) and ranged from 79.33 to 17.25. ANCOVAs between

Table 3. Number, mean shell length (mm ± *SE*), and male to female ratio of four whelk species collected during the entire study period and separately by each collecting period. Period 1 was February 27 – March 06, Period 2 was March 13 – March 16, and Period 3 was March 26 – April 01. Not all specimens were sexed. Only one pearwhelk measuring 85 mm was found. A goodness-of-fit *G*-test was calculated for the number of males to females to identify those significantly different from a 1:1 ratio (*, $P < 0.05$; **, $P < 0.01$).

	Total	Period 1	Period 2	Period 3
Knobbed whelk				
Number	1,663	968	485	210
All shell length	121.59 ± 0.79	129.58 ± 1.00	111.70 ± 1.33	107.61 ± 2.28
Female shell length	131.29 ± 0.86	136.65 ± 1.00	121.44 ± 1.72	116.25 ± 2.96
Male shell length	87.80 ± 0.79	91.24 ± 1.09	87.69 ± 1.22	82.29 ± 2.06
M:F sex ratio	0.24**	0.19**	0.33**	0.38**
Channeled whelk				
Number	86	38	22	26
All shell length	81.62 ± 1.88	81.08 ± 2.92	83.86 ± 3.55	80.50 ± 3.48
Female shell length	91.62 ± 2.99	90.22 ± 3.73	101.25 ± 4.84	89.71 ± 7.36
Male shell length	86.15 ± 2.72	88.62 ± 3.12	86.20 ± 8.91	83.29 ± 3.69
M:F sex ratio	0.69	0.44*	1.25	1.00
Lightning whelk				
Number	75	50	16	8
All shell length	153.17 ± 3.59	156.46 ± 4.55	146.44 ± 6.80	154.62 ± 8.10
Female shell length	152.38 ± 3.88	155.52 ± 4.63	136.62 ± 7.04	151.20 ± 11.45
Male shell length	82.00 ± 0.00	82.00 ± 0.00		
M:F sex ratio	0.02**	0.02**	0*	0*
All whelk species				
Total number sexed	1,528	995	358	175
Number females	1,235	839	269	127
Number males	293	156	89	48
M:F sex ratio	0.24**	0.19**	0.33**	0.38**

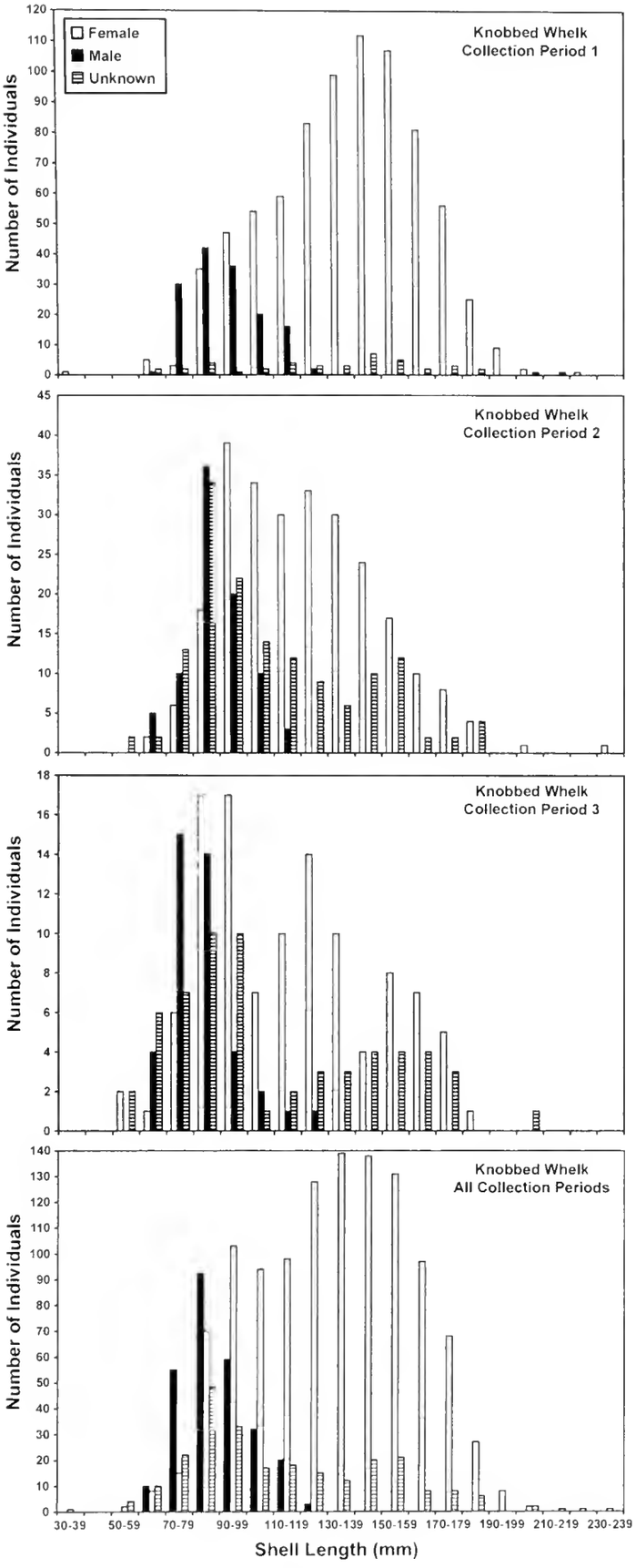

Figure 6. Size frequency distributions (shell length in mm) for male, female, and unsexed knobbed whelks harvested during the three collection periods (Feb. 27 to Mar. 06, Mar. 13 to Mar. 16, and Mar. 26 to Apr. 1) separately and combined.

sampling periods, using light intensity as a covariate, detected significant differences for the mean numbers caught for all whelk ($P < 0.05$) and knobbed whelk ($P < 0.001$) categories. The total number of knobbed whelk collected during the second and third harvesting periods was 50.10% and 21.69% of the first periods catch. Over a period of approximately one month, the whelk catch per unit effort decreased by 78.31%. The mean number of knobbed whelk harvested per event during the first sampling period was 74.5 and decreased to 17.5 in the third period. ANCOVAs were run between sampling periods using light intensity as the covariate, and significant differences ($P < 0.001$) were detected for the mean numbers caught for all whelk and knobbed whelk. Knobbed male to female ratios increased from 0.19 to 0.38 through the three collection periods, but remained significantly different from a 1:1 ratio ($P < 0.01$).

At the termination of sampling, the last five diurnal harvesting events returned ten or fewer whelk on each occasion. Within two weeks our nocturnal efforts would similarly have declined (Fig. 8). The decrease in the catch per unit effort of all and knobbed whelk according to night and day intervals is charted (Fig. 9). The changes in the mean number of individuals for the night and day intervals corresponds to the quadrants from sunrise and to sunset (0 to +2), from sunset and to sunrise (0 to -2), and those representing the middle of the day (+2 to +4) and the middle of the night (-2 to -4). The fastest rate of reduction was observed for the middle of the night samplings, followed by the middle of the day, and then the hours both before sunrise and after sunset, and finally the hours after sunrise and before sunset (Table 4). We estimate that in coastal Georgia, an intertidal whelk population inhabiting an approx. area of 2.4 ha (450 m × 60 m) can be completely fished out in about six weeks.

DISCUSSION

Previous studies have also documented dominance by the knobbed whelk in the inshore waters of Georgia and South Carolina (Anderson *et al.* 1985, Power *et al.* 2002a, Walker *et al.* 2008). Similar to most sexually dimorphic, commercial whelk species, female whelk at Dead Man Hammock attained a larger size than males in all species (MacIntosh and Paul 1977, Anderson *et al.* 1985, Kideys *et al.* 1993, Castagna and Kraeuter 1994, Power and Keegan 2001, Bruce *et al.* 2006, Walker *et al.* 2008, Power *et al.* 2009). Our data also support previous findings in Wassaw Sound for the size ranges of the various species of whelk in intertidal habitats, and rank them from largest to smallest as follows: lightning, knobbed, channeled, and pear (Walker *et al.* 2008). Our sample size of lightning whelk was small; however, these snails appeared to exhibit a smaller size than those from the

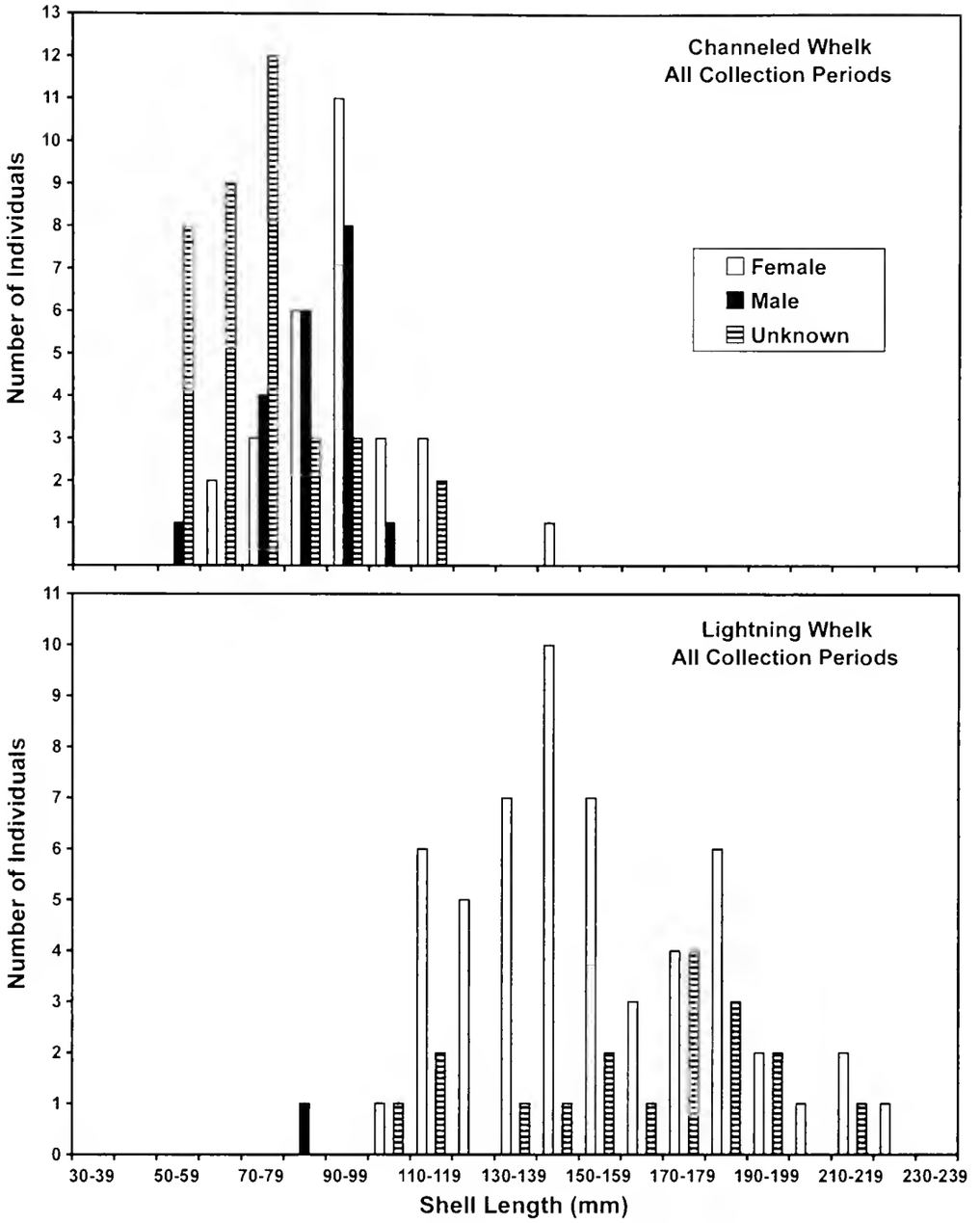

Figure 7. Size frequency distributions (shell length in mm) for male, female, and unsexed channeled and lightning whelks harvested during the three collection periods (Feb. 27 to Apr. 1).

Gulf Coast of Florida (Menzel and Nichy 1958, Kent 1983), supporting Berlocher's (2000) theory of distinct subspecies or unresolved species between the South Atlantic coast and the Gulf of Mexico. In agreement with other studies, the whelk species on Dead Man Hammock were also heavily dominated by females (Power *et al.* 2002b, Walker *et al.* 2008). Our male to female ratios mirror those reported by Walker *et al.* (2008), with the fewest males occurring in lightning whelk, more males in knobbed whelk, and the most males occurring in channeled whelk, though never approaching parity.

Higher abundances of whelk in intertidal habitats in spring and fall in the southeastern United States have been attributed to migrations to and from subtidal waters; periods of quiescence in the summer and winter suggest whelk are largely buried on the sand/mud flats (Magalhaes 1948, Walker 1988, Walker *et al.* 2004, Shalack 2007). Oysters and clams are the most important prey species for the dominant intertidal species, the knobbed whelk (Carriker 1951, Paine 1962, Davis 1981, Walker 1988, Ferner and Weissburg 2005, Shalack 2007, Walker *et al.* 2008). These bivalves occur predominantly in the intertidal creeks and sounds in coastal Georgia (Harris 1980, Walker and Tenore 1984). During the winter and summer months, environmental conditions in the exposed intertidal zone are extremely harsh, which may reduce feeding rates. The

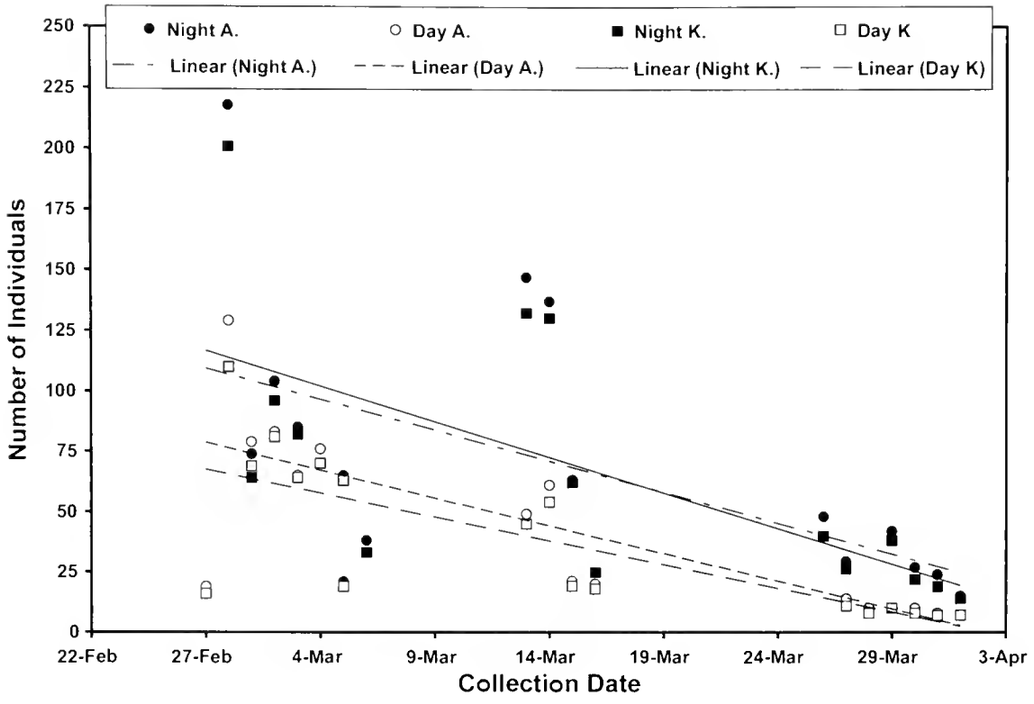

Figure 8. The reduction in the nocturnal and diurnal catch per unit effort for all intertidal whelks and specifically for knobbed whelk over the entire study period (Feb. 27 to Apr. 1). Nocturnal collections occurred between sunset and sunrise.

knobbed whelk has been observed to bury in place and remain inactive at temperatures below 14 °C (Walker *et al.* 2004), and it is likely that similar behavior occurs when excessive temperatures could cause desiccation. Polites and Mangum (1980) noted channeled whelk sluggishness at temperatures greater than 24 °C. Ferner and Weissburg (2005) have observed knobbed whelk feeding on clams and oysters at night in August, but noted their complete absence during the day.

Whelk are readily observed feeding, copulating, and egg laying on these same flats during diurnal low tide exposures in the spring and fall. In Georgia, male knobbed whelk become sexually mature at 85-90 mm, or four years of age, and approx. 100 mm or six years when female (Power *et al.* 2009). Sex ratios can vary geographically and seasonally (Magalhaes 1948, Weinheimer 1982, Anderson *et al.* 1985, Castagna and Kraeuter 1994, Eversole *et al.* 2008, Walker *et al.* 2008). Fertilization in all species is internal after multiple matings, and direct development to juveniles measuring 3 to 7 mm is completed in weeks to months within egg capsules attached along an egg-case string (Castagna and Kraeuter 1994, Power *et al.* 2002a). Anchored strings from all species have been documented from Georgia's intertidal sandy mud flats and subtidal waters in both spring and fall months (Walker 1988, Power *et al.* 2002a, 2009, Walker *et al.* 2008). It is doubtful, given energetic requirements, whether the same females spawn in both seasons.

Given the limited mobility of knobbed whelk (Magalhaes 1948, Ferner 2006, Shalack 2007, Walker *et al.* 2008), intertidal populations are probably localized and remain year around, but confine feeding during warmer summer and colder winter months to the high tide stage and night time. Spring and fall provide the optimal exposure conditions, which may control reproductive cycles since the deposition of eggs strings can take many days and tidal cycles to complete (Power *et al.* 2002a). These same factors may explain the diurnal and nocturnal abundance patterns observed for knobbed and channeled whelk in the present study: whelk were most active at night (or high tide), but also emerged during diurnal exposure, due to the time of year. Different diurnal behavior patterns have also been reported for whelk from other geographical areas. Magalhaes (1948) found channeled whelk from North Carolina to be more active at night and Paine (1962) reported that pearwhelk actively fed at night in the laboratory. We did not identify any relationship between light intensity and lightning whelk abundance, which corroborates findings from Florida where they are more common and active at all hours (Paine 1962, Kent 1983). Lightning whelk have the largest shells and therefore may be less vulnerable to predation and desiccation than the knobbed, channeled, or pear whelk.

Hand harvesting rapidly reduced the mean size and depleted the numbers of whelk in the study area. We estimate that hand harvesting could remove whelk stocks from a sand/mud flat of approx. 2.4 ha in as short a time as six weeks during a period when whelk activity is at its peak. Therefore, we conclude that a commercial fishery would not be

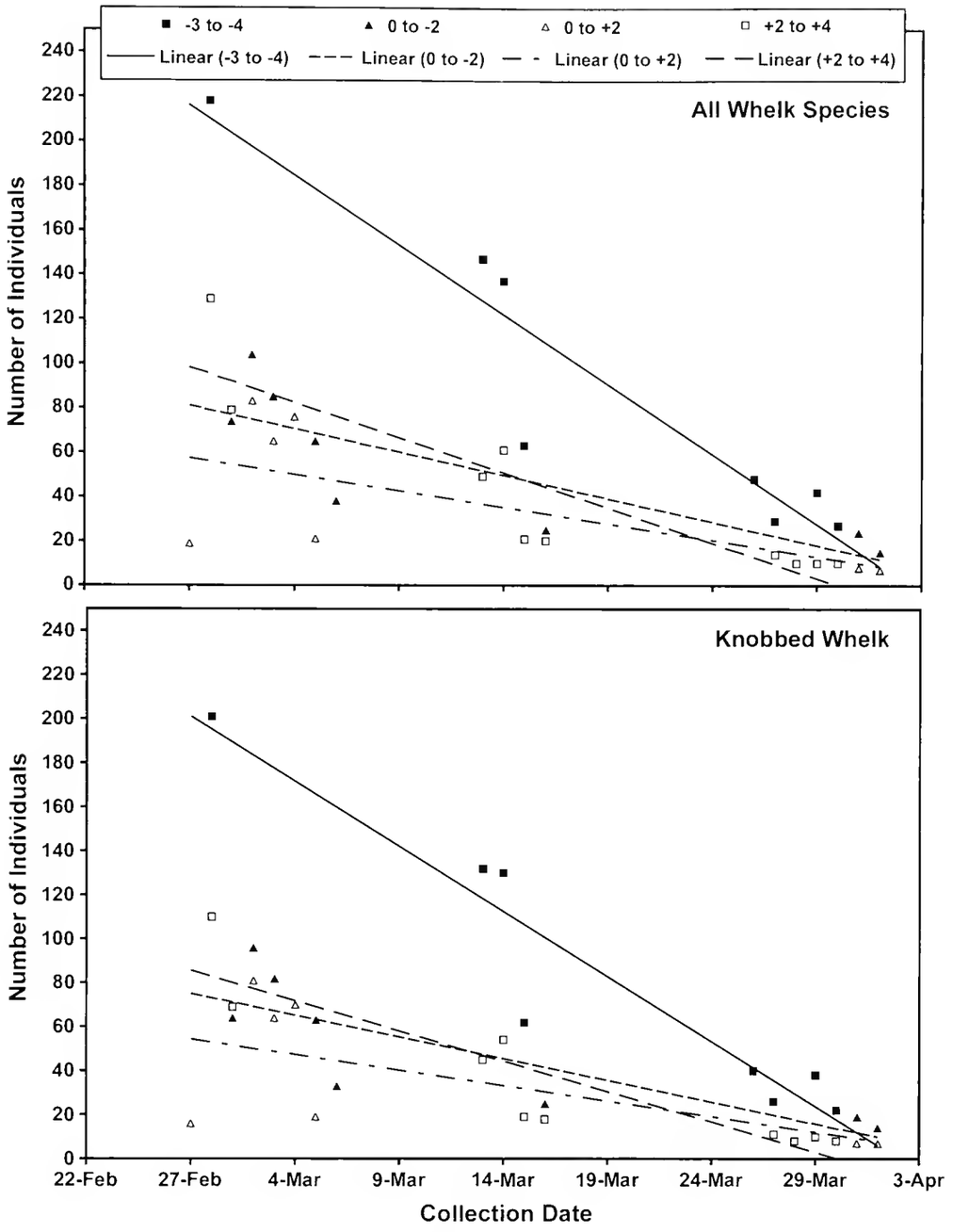

Figure 9. The reduction in the catch per unit effort for all intertidal whelks and specifically for knobbed whelk, according to the mean number of individuals for the day intervals. Day intervals correspond to the quadrants from sunrise and to sunset (0 to +2), from sunset and to sunrise (0 to -2), and those representing the middle of the day (+2 to +4) and the middle of the night (-2 to -4).

sustainable for very long in intertidal locations. In addition, these periods of peak abundances also coincide with the time leading up to the spawning season. While whelk could be very efficiently captured in a seasonal, night-based hand picking fishery, this would predominantly remove large females from the population before they could reproduce and would have detrimental impacts on recruitment to local populations. Whelk have limited mobility and grow slowly (Kraeuter *et al.* 1989, Walker *et al.* 2008), mature at a late age (Power *et al.* 2009), and have low juvenile hatching rates (Power *et al.* 2002b), making them particularly vulnerable to over-exploitation. The gathering of blue crabs, hard clams, oysters, and penaeid shrimp is prohibited by Georgia Department of Natural Resources at night to prevent poaching from non-approved areas, and we suggest this restriction should be applied to protect intertidal whelk populations during the spring and fall months. As an alternative to being closed completely during these months, the enforcement of a catch limit would greatly benefit the sustainability of resources. Outside of the spawning seasons, the catch per unit effort

Table 4. Equations of lines (y = ax + c) fitted to Figs. 8 and 9 to describe the decrease in numbers of individual knobbed and all whelks harvested over the study period in relation to the collection time of day. Time of day is expressed as night or day and also in terms of quadrants (from sunrise and to sunset, 0 to +2; from sunset and to sunrise, 0 to -2; and those representing the middle of the day, +2 to +4, and the middle of the night, -2 to -4).

Night and day	a	c	R^2
Knobbed night	-2.94	114,317	0.43
Knobbed day	-1.97	76,339	0.57
All night	-2.57	99,595	0.30
All day	-2.30	89,273	0.62
All whelk	a	c	R^2
-3 to -4	-6.29	244,162	0.89
0 to -2	-2.10	81,562	0.68
0 to +2	-1.49	57,705	0.40
+2 to +4	-3.18	123,233	0.79
Knobbed whelk	a	c	R^2
-3 to -4	-5.91	229,471	0.90
0 to -2	0.29	-11,214	0.01
0 to +2	2.54	-98,518	0.35
+2 to +4	0.89	-34,455	0.10

could be enhanced to support a supplemental fishery by collecting at night.

ACKNOWLEDGMENTS

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Appendix 1. Number of individuals of whelk collected in relation to date, time, sunrise and sunset, day interval (four quadrants from sunrise to midday ranked 0 to 4, quadrants from midday to sunset ranked 4 to 0, quadrants from sunset to the middle of night ranked 0 to -4, and quadrants from middle of night to sunrise ranked -4 to 0), MLLW Mean Lower Low Water (NOAA, Beach Hammock), percentage of moon visible (US Naval Observatory), water and air temperature (thermometer at Dead Man Hammock).

Date	Start	End	Sunrise	Sunset	Light intensity	Tidal height	Low tide	Lunar phase	Water temp.	Air temp.	Knob.	Light.	Chan.	All whelk
M/D/Y	(h:min)	(h:min)	(h:min)	(h:min)	(-4 to 4)	(MLLW)	(h:min)	(% Visible)	(°C)	(°C)	(#)	(#)	(#)	(#)
2/27/2006	15:00	16:40	6:54	18:21	2.5	-0.37	13:41	0	13.5	14	16	3	0	19
2/28/2006	1:30	4:45	6:53	18:21	-3	-0.43	1:58	1	12.5	9	201	9	8	218
2/28/2006	14:00	16:20	6:53	18:21	3	-0.46	14:29	1	13	18.5	110	16	3	129
3/1/2006	2:40	4:45	6:52	18:22	-2.5	-0.46	2:49	4	13	11	64	2	8	74
3/1/2006	14:10	16:00	6:52	18:22	3	-0.46	15:15	4	14.5	23	69	9	1	79
3/2/2006	3:10	4:55	6:51	18:23	-2.5	-0.40	3:39	10	14	13	96	2	6	104
3/2/2006	14:35	16:15	6:51	18:23	2.5	-0.43	16:00	10	15.5	24	81	2	0	83
3/3/2006	3:45	5:25	6:49	18:24	-1.5	-0.27	4:29	19	15.5	16	82	0	3	85
3/3/2006	16:10	17:30	6:49	18:24	2	-0.30	16:46	19	16.5	14.5	64	1	0	65
3/4/2006	16:35	18:05	6:48	18:24	1.5	-0.15	17:33	28	15.5	11.5	70	3	3	76
3/5/2006	6:05	7:15	6:47	18:25	0	0.09	6:12	38	14	8	63	1	1	65
3/5/2006	17:30	18:35	6:47	18:25	1	0.00	18:24	38	15	13	19	1	1	21
3/6/2006	7:05	8:10	6:46	18:26	-1.5	0.24	7:12	50	14.5	12	33	1	4	38
3/13/2006	:20	2:20	6:37	18:31	-4	0.06	:55	99	18	18	132	8	7	147
3/13/2006	12:30	14:00	6:37	18:31	4	0.09	13:17	99	18.5	24.5	45	3	1	49
3/14/2006	:40	2:30	6:36	18:32	-3.5	0.03	1:36	100	18.5	18.5	130	1	6	137
3/14/2006	13:15	14:45	6:36	18:32	4	0.06	13:52	100	19	21	54	1	6	61
3/15/2006	1:20	3:05	6:35	18:32	-3.5	0.00	2:14	100	18	11.5	62	0	1	63
3/15/2006	13:30	14:35	6:35	18:32	4	0.03	14:25	100	17.5	18.5	19	2	0	21
3/16/2006	2:40	4:10	6:33	18:33	-2.5	0.00	2:51	97	17	10	25	0	0	25
3/16/2006	14:25	15:40	6:33	18:33	3	0.03	14:57	97	17	19.5	18	1	1	20
3/26/2006	23:05	:55	6:21	18:40	-4	-0.15	23:51	11	13.5	9	40	1	7	48
3/27/2006	11:40	13:00	6:19	18:41	4	-0.24	12:25	4	13.5	14.5	11	3	0	14
3/27/2006	:10	1:30	6:19	18:41	-4	-0.27	:48	1	13.5	11	26	1	2	29
3/28/2006	12:15	13:25	6:18	18:41	4	-0.34	13:14	1	14.5	19.5	8	2	0	10
3/29/2006	1:00	2:30	6:17	18:42	-3.5	-0.34	1:41	0	14.5	14.5	38	0	4	42
3/29/2006	13:10	14:30	6:17	18:42	4	-0.40	14:02	0	15.5	21.5	10	0	0	10
3/30/2006	1:45	3:15	6:15	18:43	-3.5	-0.34	2:33	2	15.5	15.5	22	1	4	27
3/30/2006	13:55	15:25	6:15	18:43	3.5	-0.40	14:28	2	16	17	8	0	2	10
3/31/2006	2:40	4:00	6:14	18:43	-2.5	-0.30	3:22	7	15.5	15.5	19	0	5	24
3/31/2006	14:55	16:20	6:14	18:43	2.5	-0.30	15:34	7	17.5	24	7	0	1	8
4/1/2006	3:30	5:00	6:13	18:44	-2	-0.18	4:10	14	17	16.5	14	0	1	15
4/1/2006	15:15	16:30	6:13	18:44	2	-0.18	16:19	14	18.5	23.5	7	0	0	7

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Multi-data set revision of two uncommon species of Chromodorididae (Nudibranchia) from the Gulf of Mexico

Ángel Valdés[1], Ulysses Gatdula[1], Nancy Sheridan[2], and Joan Herrera[2]

[1]Department of Biological Sciences, California State Polytechnic University, 3801 West Temple Avenue, Pomona, California 91768, U.S.A.
[2]Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Avenue SE, St. Petersburg, Florida 33701, U.S.A.

Correspondence, A. Valdés: aavaldes@csupomona.edu

Abstract: Morphological and molecular data are used to address the taxonomic status of two uncommon species of opisthobranch molluscs from the Gulf of Mexico. *Chromodoris fentoni* is a new species closely related to other Atlantic and eastern Pacific congeneric species. It is characterized by having a whitish background color almost completely covered with irregular red pigment. *Glossodoris punctilucens* Bergh, 1890 is a rare species known from only a handful of specimens. Morphological and molecular data confirm that it is distinct from other similarly colored species from the eastern Atlantic.

Key words: *Chromodoris*, *Glossodoris*, morphology, COI, H3

Several species of Chromodorididae have been described from the Caribbean and Gulf of Mexico. Most of the historic descriptions are based on preserved material and therefore lack information on the color of the living animals (Valdés *et al.* 2006). Modern publications generally include both descriptions of the living animals and anatomical data (Valdés *et al.* 2006), greatly improving the possibility of other authors identifying these species. However, it has become evident that color and anatomical information alone are not always sufficient to provide a solid framework for solving complex taxonomic issues involving closely related species. Pola *et al.* (2006) illustrated how molecular information combined with anatomical data can be used to effectively address problems of color variation and species boundaries in an eastern Pacific opisthobranch species complex, opening the door for applying this methodology to other groups of species.

In this paper, we attempt to clarify the systematics of two uncommon species using a combination of external morphology, anatomy, biology, and sequence data from a mitochondrial and a nuclear gene, and comparing this information with other closely related species.

MATERIALS AND METHODS

Collection and preservation

The specimens were collected from the Gulf of Mexico, west Florida shelf. This area consists of a hard-bottom habitat of low relief carbonate structures, commonly called reef ledges. These discontinuous ledges are generally oriented north-south, rise from less than one meter to several meters off the sandy bottom, and support a diverse community of marine organisms. On 30 March 2009, while scuba diving at a depth of 9 m, commercial aquarium trade fisher, Daniel Fenton, collected by hand a specimen of the red sponge *Igernella notabilis*, which contained two specimens of *Chromodoris fentoni*. The sponge and nudibranchs were placed into plastic bags with seawater and transported to the Florida Fish and Wildlife Conservation Commission's (FWC), Fish and Wildlife Research Institute (FWRI). Features of these living organisms were digitally photographed in a photographic plexiglass aquarium at the FWRI.

On two subsequent field trips with Daniel Fenton, FWRI scientific research scuba divers, at a depth of 9-10 m, searched along ledges for *Igernella notabilis* where *Chromodoris fentoni* had been previously collected. While conducting the search for *C. fentoni* on 21 June 2009, *Glossodoris punctilucens* Bergh, 1890 was found traversing the substrate. On 21 July 2009, *C. fentoni* was found on *I. notabilis*. On each occasion, FWRI scientific divers digitally photographed the specimens *in situ* and then collected them by hand, placed them into a plastic bag while underwater, and then transferred them from the bag into a five gallon bucket of seawater for transport back to the FWRI. While at the FWRI, the specimens were digitally photographed in a photographic plexiglass aquarium. During subsequent field trips by D. Fenton more specimens of *G. punctilucens* were collected: on 28 January 2010 two specimens were found on an unidentified sponge and on 3 June 2010 two more specimens were collected on the same species of sponge. This time the sponge was collected and tentatively identified as *Ircinia* cf. *campana*. On a separate occasion D. Fenton (pers. comm.) observed *G. punctilucens* feeding on

this sponge species and the nudibranchs left feeding markings as they moved on the surface of the sponge. All collected specimens were fixed and preserved in 95% ethanol to facilitate genetic analyses. All specimens were catalogued and accessioned, and were deposited in the FWRI Specimen Information Services (SIS) collection of the Florida Fish and Wildlife Conservation Commission - Fish and Wildlife Research Institute (abbreviated as FSBC).

Morphological examination

Preserved specimens were dissected and the internal features were examined and drawn using a dissecting microscope (Nikon SMZ-100) with the aid of a *camera lucida* attachment. The buccal mass of one individual of each species was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue. The radula and jaw were then rinsed in water, dried, mounted, and sputter coated for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the Natural History Museum of Los Angeles County.

DNA extraction

In addition to the specimens collected for this study, several other species were sequenced because of their color similarity with *Chromodoris fentoni*. These include *Chromodoris rolani* Ortea, 1988, *Chromodoris sphoni* (Ev. Marcus, 1971), and *Glossodoris baumanni* (Bertsch, 1970) (Table 1). DNA extraction was performed using either a hot Chelex® protocol or the DNeasy Blood and Tissue Kit (Qiagen). Approximately 1-3 mg of the foot was cut into fine pieces for extraction for both protocols. For the Chelex extraction, the foot tissue was rinsed and rehydrated using

Table 1. List of specimens used in the molecular study, including locality, voucher number, and GenBank Accession numbers. Abbreviations: FSBC, Florida Fish and Wildlife Conservation Commission - Fish and Wildlife Research Institute; SAM, South Australian Museum, Adelaide; LACM, Natural History Museum of Los Angeles County.

			GenBank Accession No.	
Species	Locality	Voucher No.	H3	COI
Chromodoris alternata	-	SAM D19281	-	EF535120
C. ambiguus	-	SAM D19260	-	EF535119
C. epicuria	-	SAM D19285	-	EF535114
C. fentoni	Pinellas County, FL	FSBC I 67095	GU815542	GU815540
C. krohni	-	-	-	AF249805
C. krohni	-	-	-	AY345036
C. kuiteri	-	-	-	AF249804
C. kuniei	-	SAM D19261	-	EF535112
C. leopardus	-	SAM D19288	-	EF535116
C. luteorosea	-	-	-	AJ223259
C. magnifica	-	SAM D19290	-	EF535110
C. purpurea	-	-	-	AJ223260
C. quadricolor	-	-	-	AF249802
C. rolani	Cape Verde	LACM 153124	GU815543	GU815541
C. sphoni	Huatulco, Mexico	-	GU815544	-
C. sphoni	-	LACM 175026	GU815545	-
C. splendida	-	SAM D19292	-	EF535115
C. striatella	-	SAM D19293	-	EF535111
C. tasmaniensis	-	SAM D19295	-	EF535113
Glossodoris atromarginata	-	-	-	AF249789
G. baumanni	Huatulco, Mexico	-	GU815546	-
G. cincta	-	-	-	EF535136
G. edmundsi	-	-	-	EF535133
G. pallida	-	-	-	EF535138
G. pullata	-	-	-	EF535137
G. punctilucens	Pinellas County, FL	FSBC I 67201	GU815547	GU815539
G. sedna	-	-	-	EF535134
G. sibogae	-	-	-	EF535135
Noumea haliclona	-	SAM D19269	-	EF535117

1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 minutes. A 10% (w/v) Chelex 100 (100-200 mesh, sodium form, Bio-Rad) was prepared using TE buffer. After re-hydration, the mixture was then centrifuged, 975.00 µL of the supernatant was removed, and 175.00 µL of the Chelex solution was added. Samples were then heated in a 56 °C water bath for 20 minutes, heated in a 100 °C heating block for 8 minutes, and the supernatant was used for PCR. The DNeasy protocol supplied by the manufacturer was followed, with some modifications. The elution step was modified such that the first elution was collected using 100.00 µL of Buffer AE and was allowed to incubate at room temperature for 5 minutes. In a new test tube, a second elution step was conducted using 200.00 µL of Buffer AE and was also allowed to incubate at room temperature for 5 minutes. The first elution was used for PCR.

PCR amplification and sequencing

Colgan's universal H3 primers (Colgan *et al.* 1998) were used with all specimens to amplify the region of interest. Folmer's universal COI primers (Folmer *et al.* 1994) were used to amplify the regions of interest for all specimens (Table 1).

The master mix was prepared using 34.75 µL H_2O, 5.00 µL Buffer B (ExACTGene, Fisher Scientific), 5.00 µL 25 mM $MgCl_2$, 1.00 µL 40mM dNTPs, 1.00 µL 10mM primer 1, 1.00 µL primer 2, 0.25 µL 5 mg/mL Taq, and 2.00 µL extracted DNA. Reaction conditions for H3 were as follows: an initial denaturation for 2 min at 94 °C, 35 cycles of (1) denaturation for 30 sec at 94 °C, (2) annealing for 30 sec at 50 °C, and (3) elongation for 1 min at 72 °C, and a final elongation for 7 min at 72 °C. Reaction conditions for COI involved an initial denaturation for 2 min at 94 °C, 35 cycles of (1) denaturation for 30 sec at 94 °C, (2) annealing for 30 sec at 50°C, and (3) elongation for 1 min at 72 °C, and a final elongation for 7 min at 72 °C.

PCR products yielding bands of appropriate size (approx. 375 bp for H3 and 700 bp for COI) were purified using the Montage PCR Cleanup Kit (Millipore). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 2.0 pmol/µL to send out for sequencing with the PCR products. PCR products were diluted to 6.0, 7.5, and 11.5 ng/µL for H3 and COI, respectively. Samples were sequenced at the City of Hope DNA Sequencing Laboratory (Duarte, California) using chemistry types BigDye V1.1 for fragments less than 500 bp and BigDye V3.1 for fragments larger than 500 bp.

Phylogenetic analyses

Sequences for each gene were assembled and edited using Geneious Pro 4.7.4 (Biomatters Ltd.). Geneious was also used to extract the consensus sequence and to construct the alignment for each gene using the default parameters. The sequences were not trimmed after alignment. A total of 328 bp for H3 and 658 bp for COI were used for the phylogenetic analyses. Analyses were conducted for species of *Chromodoris* and *Glossodoris* separately and together, using COI data and including sequences available in GenBank (Table 1). Additionally, an analysis of all the H3 available sequences was conducted. For the *Glossodoris* COI analysis, *Chromodoris fentoni* was selected as the outgroup and for the *Chromodoris* COI and H3 analyses, *Glossodoris punctilucens* was selected as the outgroup. For the combined *Glossodoris* + *Chromodoris* COI analysis, *Noumea haliclona* (Burn, 1957) was selected as the outgroup.

The levels of saturation for each gene and for the first and second versus third codon positions of COI and H3 were

Table 2. Summary of each data set used for analysis with the best-fit evolutionary models and estimated parameters.

Parameters	H3	*Chromodoris* COI	*Glossodoris* COI	*Chromodoris* + *Glossodoris* COI
No. of specimens used	6	18	9	25
No. of included characters	328	587	587	587
Best-fit model	TrN+I	GTR+I+G	TIM2+I+G	GTR+I+G
Frequency A	0.2468	0.3046	0.2511	0.3080
Frequency C	0.3041	0.0945	0.1108	0.0857
Frequency G	0.2545	0.1606	0.1820	0.1621
Frequency T	0.1946	0.4403	0.4560	0.4441
R-matrix [A-C]	1.0000	1.6928	0.0730	1.5579
R-matrix [A-G]	5.4423	23.4219	9.8678	19.6718
R-matrix [A-T]	1.0000	1.0448	0.0730	0.6047
R-matrix [C-G]	1.0000	11.8056	1.0000	11.3414
R-matrix [C-T]	13.0475	166.4768	76.9023	170.8606
R-matrix [G-T]	1.0000	1.0000	1.0000	1.0000
Γ shape (G)	-	0.5470	0.1850	0.2680
Proportion of Invariant Sites (I)	0.7840	0.5660	0.4240	0.4607

investigated using the substitution saturation test developed by Xia *et al.* (2003) and Xia and Lemey (2009) implemented in the program DAMBE (Xia and Xie 2001).

The Akaike information criterion (Akaike 1974) was executed in jModelTest (Posada 2008) to determine the best-fit model of evolution (Table 2). Maximum likelihood analyses were conducted using PAUP*4.0 (Swofford 2002). Robustness of each clade was assessed by bootstrap support (Felsenstein 1985) based on 2000 replicates with heuristic search, TBR branch-swapping algorithm, multrees option, 100 random additions. For the combined *Chromodoris* + *Glossodoris* COI analysis only 1 random addition for each bootstrap replicate was investigated due to computer power constraints.

SPECIES DESCRIPTIONS

Chromodoris fentoni new species
(Figs. 1A-B, 2-3)

Material examined

Holotype: off Pinellas County, Florida (28.17100°N, 83.00200°W), 30 March 2009, 9 m depth, specimen 15 mm preserved length, leg. D. Fenton (FSBC I 67094). Paratypes: off Pinellas County, Florida (28.17100°N, 83.00200°W), 30 March 2009, 9 m depth, 1 specimen 16 mm preserved length, dissected, leg. D. Fenton (FSBC I 67095). Off Pinellas County, Florida (28.07076°N, 82.54663°W), 21 July 2009, 9 m depth, 1 specimen 8 mm long, leg. N. Sheridan (FSBC I 67253).

External morphology

The body has a whitish background color almost completely covered with irregular red pigment (giving the animal a reddish appearance) and many yellow spots with an orange center (Fig. 1A). The yellow-orange spots are more densely arranged on the mantle margin. The mantle edge is surrounded by a translucent grayish band, followed by a whitish band, and a narrow yellow line. The branchial leaves (Fig. 1B) and rhinophores are white with a few reddish spots and a submarginal reddish patch on each leaf and rhinophore; the apices of the leaves and rhinophores are opaque white.

There are 10 branchial leaves surrounding the anus. The mantle margin contains two disorganized bands of small, discrete, oval mantle glands.

Reproductive system

The reproductive system contains a long, narrow ampulla that connects to the prostate and the female gland complex (Fig. 2B). The prostate is long and convoluted and narrows into a long, muscular deferent duct that expands into the broad muscular portion of the deferent duct (Fig. 2A). The vagina is short, slightly convoluted and connects directly into the large, irregular bursa copulatrix. The seminal receptacle is elongate, and also connects directly into the bursa copulatrix at some distance from the vaginal insertion. The straight uterine duct leaves the bursa copulatrix next to the insertion of the seminal receptacle and inserts into the female gland complex, near the opening of the ampulla.

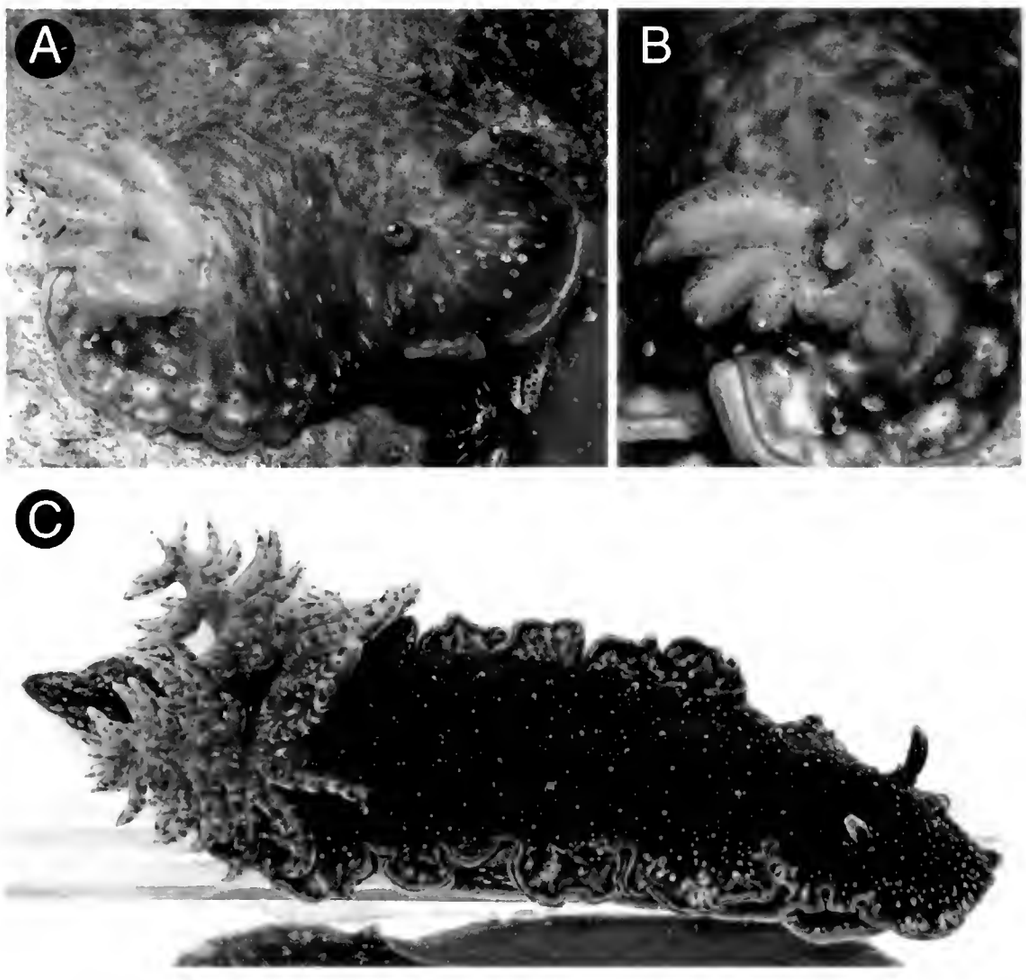

Figure 1. Photographs of living animals. A, Aquarium photograph of the holotype of *Chromodoris fentoni* (FSBC I 67094) by M. Colella; B, Detail of the gill of the holotype of *Chromodoris fentoni* (FSBC I 67094) by M. Colella; C, Aquarium photograph of *Glossodoris punctilucens* (FSBC I 67201) by N. Sheridan.

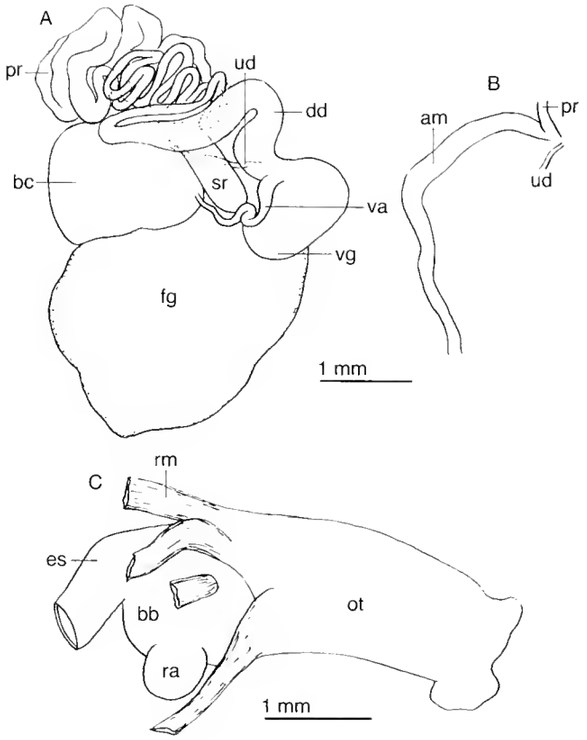

Figure 2. Drawings of the anatomy of the paratype of *Chromodoris fentoni* (FSBC I 67095). A, Reproductive system; B, Detail of the ampulla; C, Anterior portion of the digestive system. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; dd, deferent duct; es, esophagus; fg, female gland complex; ot, oral tube; pr, prostate; ra, radular sac; rm, retractor muscle; sr, seminal receptacle; ud, uterine duct; va, vagina; vg, vestibular gland.

Digestive system

The buccal bulb is relatively short, about three times shorter than the oral tube (Fig. 2C). The radular formula is 54 × 23.0.23 in the paratype. There are no rachidian teeth. The innermost lateral teeth are wider than the rest of the laterals (Fig. 3A), and have an elongate cusp with 1-2 inner denticles and 3-4 outer denticles. The mid lateral teeth are hook-shaped with 6-8 denticles (Fig. 3C). The outer laterals have 3-6 denticles (Fig. 3B). The jaw consists of numerous bicuspid rodlets (Fig. 3D).

Molecular data

Chromodoris fentoni is genetically distinct from other species of *Chromodoris* for which we have molecular data. The data sets available are too incomplete to say anything relevant about the phylogenetic relationships of this species but allow us to compare it with other morphologically closely related species. For example, the most similar species in regard to the external coloration are the eastern Pacific *Glossodoris baumanni* (Bertsch, 1970) and *Chromodoris sphoni* (Ev. Marcus, 1971), which show sequence divergences of 4.8% and 2.4%, respectively, with *C. fentoni* in the H3 gene.

Biology

All specimens were collected on the red sponge *Igernella notabilis* (Demospongiae: Ceractinomorpha: Dendroceratida: Dictyodendrillidae), at 9 m depth, which most likely constitutes their diet. The sponge specimen is deposited at the FWRI collections (FSBC I 67300). This species is known only from the Gulf of Mexico.

Etymology

The species is named after Daniel Fenton (Florida), the collector of the original type specimens.

Remarks

The generic placement of *Chromodoris fentoni* is problematic as this species displays characteristics of both *Chromodoris* and *Glossodoris* according to the diagnoses by Rudman (1984). For instance, the double row of mantle glands and the long oral tube in comparison to the buccal bulb are characteristics of members of *Glossodoris* (Rudman 1984). However, the radula of *C. fentoni* is relatively short and wide, protruding as a short radular sac, the body profile is relatively low and wide, the gill is composed of leaves arranged in a semi-circle. All these characteristics are consistent with Rudman's (1984) diagnosis of *Chromodoris* and the features found in other western Atlantic species of this group. More importantly, molecular data reveal that *C. fentoni* is more closely related to *Chromodoris krohni* (Vérany, 1946) than to any species of *Glossodoris*.

Chromodoris fentoni is clearly distinguishable from other Atlantic and Eastern Pacific species of *Chromodoris*. The most similar species in external coloration is the Eastern Pacific *Glossodoris baumanni* (Bertsch, 1970), which also has a light background color with irregular red pigment, and whitish rhinophores and gills with subapical reddish pigment (Gosliner *et al.* 2004). However, *G. baumanni* lacks the characteristic yellow-orange spots present in *C. fentoni*. Anatomically, these two species are very different as *G. baumanni* has very elongate mid-lateral teeth with the denticles concentrated near the end of the cusp (Gosliner *et al.* 2004), whereas in *C. fentoni* the mid-lateral teeth are shorter and the denticles more evenly distributed. In *G. baumanni* the rachidian row of teeth may be present or absent. The reproductive system of *G. baumanni* has a highly developed deferent duct and the uterine duct is partially fused with the vagina (Gosliner *et al.* 2004), both characteristics are absent in *C. fentoni*. Incidentally, Gosliner *et al.* (2004) transferred *G. baumanni* to the genus *Glossodoris*, but preliminary molecular data here presented seem to suggest that the original placement in *Chromodoris* (Bertsch 1970) was correct although support levels are too low to reach a definitive conclusion.

Another Eastern Pacific species, *Chromodoris sphoni* (Ev. Marcus, 1971), has similar rhinophore and gill coloration to

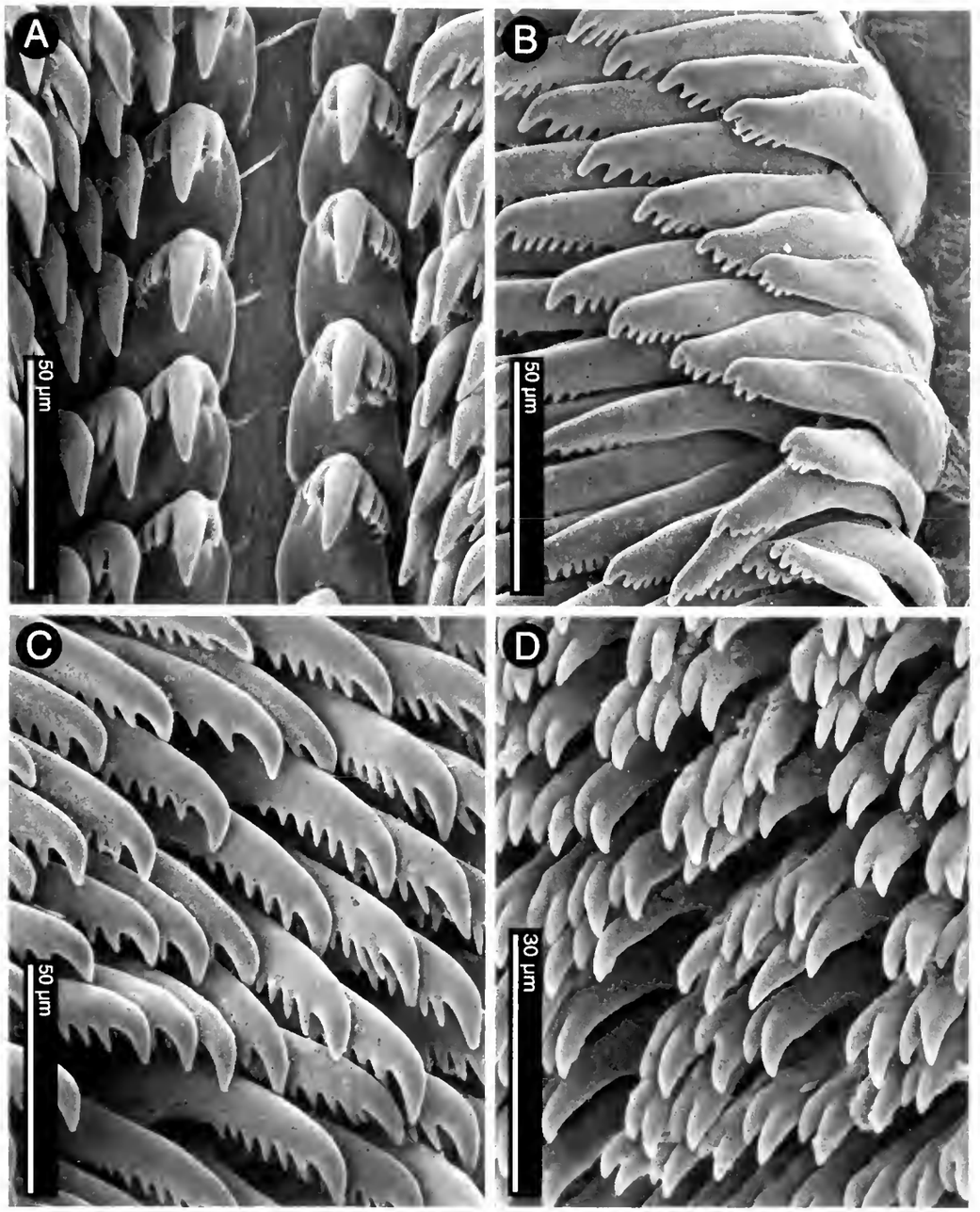

Figure 3. SEM micrographs of the radula and jaws of the paratype of *Chromodoris fentoni* (FSBC I 67095). A, Innermost lateral radular teeth; B, Outermost lateral radular teeth; C, Mid-lateral radular teeth; D, Jaw rodlets.

C. fentoni, and also has dorsal yellow spots, but the color pattern is very different, having a bluish background color with a solid red cross pattern on the dorsum (Ortea *et al.* 1992). The anatomy of *C. sphoni* differs from that of *C. fentoni* as the former has a large rachidian tooth on each row, several of the innermost lateral teeth have denticles on both sides of the cusp, and the seminal receptacle is almost as large as the bursa copulatrix (Ortea *et al.* 1992).

Some Caribbean species of *Chromodoris* were described based on limited information on external coloration and anatomy. A group of species with a reticular pattern of yellow and red pigment includes *Chromodoris binza* Ev. Marcus and Er. Marcus, 1963, *Chromodoris clenchi* (Russell, 1935), and *Chromodoris neona* Er. Marcus, 1955 and was reviewed by Ortea *et al.* (1994). All these species have a large, triangular rachidian tooth on each row of the radula, which is absent in this new species. Additionally, *C. binza* and *C. clenchi* have light rhinophores and branchial leaves with well-marked purple rachises, very different from the whitish rhinophores and branchial leaves with reddish apices of this new species. *Chromodoris neona* has whitish rhinophores and branchial leaves with dark purple apices, but the body color is pale blue with a conspicuous network of bright yellow lines on the dorsum. See Valdés *et al.* (2006) for photographs of these species.

Other Caribbean species that possess a large, triangular rachidian tooth on each row of the radula and are thus easily distinguishable from this new species are *Chromodoris aila* Er. Marcus, 1961, *Chromodoris dictya* Er. Marcus and Ev. Marcus, 1970, and *Chromodoris ponga* Er. Marcus and Ev. Marcus, 1970 (Marcus 1961, Marcus and Marcus 1970). *Chromodoris aila* was described as red with blue and yellowish marks, and *C. ponga* as having a red notum surrounded by a broad white margin, whereas the original description of *C. dictya* contains no information on the color of the live animal.

Chromodoris perola Ev. Marcus, 1976 is a Caribbean species lacking rachidian teeth. The live animals were described by Marcus (1976) as having a reddish-white mantle surface, with a central row of dark, round, red spots, two lateral rows of elongated red spots, and a deep orange line around the mantle margin. The branchial leaves are opaque, and the rhinophores violet. The anatomy of *C. perola*, described by Marcus (1976), shows several important differences with that of *C. fentoni*. Although the radular morphology of these two species is similar, the jaw rodlets of *C. perola* are short and can be mono, bi, or tricuspid (Marcus 1976: fig. 25). According to the description by Marcus (1976: fig. 27) in the reproductive system of *C. perola* the seminal receptacle opens into the vagina, a short distance

from the vaginal insertion into the bursa copulatrix, and at the same point where the uterine duct opens, with all these three ducts forming a "x" pattern. This is very different from *C. fentoni* in which the vagina opens into the bursa copulatrix at some distance from where both the seminal receptacle and the uterine duct connect directly to the bursa copulatrix. *Chromodoris perola* has not been collected again since its original description and its identity cannot be verified.

More recently, two additional reddish species of *Chromodoris* were described in the Caribbean. *Chromodoris grahami* Thompson, 1980, originally described from Jamaica, is characterized by having a salmon-pink body with bright red spots and dark brown rhinophores (Thompson 1980). *Chromodoris regalis* Ortea, Caballer, and Moro, 2001 is also salmon pink with numerous white spots (Ortea *et al.* 2001). See Valdés *et al.* (2006) for photographs of these species; both are clearly distinguishable from *C. fentoni*.

Eastern Atlantic species of *Chromodoris* with a similar coloration include *Chromodoris luteorosea* (von Rapp, 1827), *Chromodoris luteopunctata* (Gantès, 1962), and *Chromodoris rolani* Ortea, 1988. All these species have a pinkish background color with yellow-orange spots on the dorsum (see Ortea 1988, Cervera *et al.* 1989, Ortea and Valdés 1992), but also have triangular rachidian teeth, which are absent in *C. fentoni*.

Glossodoris punctilucens Bergh, 1890
(Figs. 1C, 4-5)

Chromodoris punctilucens Bergh, 1890: 162-165, pl. 1, figs. 4-10.

Material examined

Off Pinellas County, Florida (28.11750°N, 82.91250°W), 21 June 2009, 9 m depth, 1 specimen 34 mm preserved length, leg. N. Sheridan (FSBC I 67201). Off Pinellas County, Florida (28.19200°N, 83.00770°W), 28 January 2010, 11 m depth, 2 specimens 14 and 15 mm preserved length, leg. D. Fenton (FSBC I 68012).

External morphology

The body is dark brown to black with a number of small white dots and larger yellow spots distributed all over the dorsum (Fig. 1C). Both white dots and yellow spots are situated at the tip of dorsal tubercles. The tubercles vary from small and conical to larger and stalked. The mantle margin is surrounded by a translucent line, followed by a thinner, irregular black line, a broader yellow line and a black-pigmented irregular area. The rest of the mantle margin bears large, oval, discrete, white mantle glands and appears blue probably because of the effect of the white mantle glands under the black epithelium.

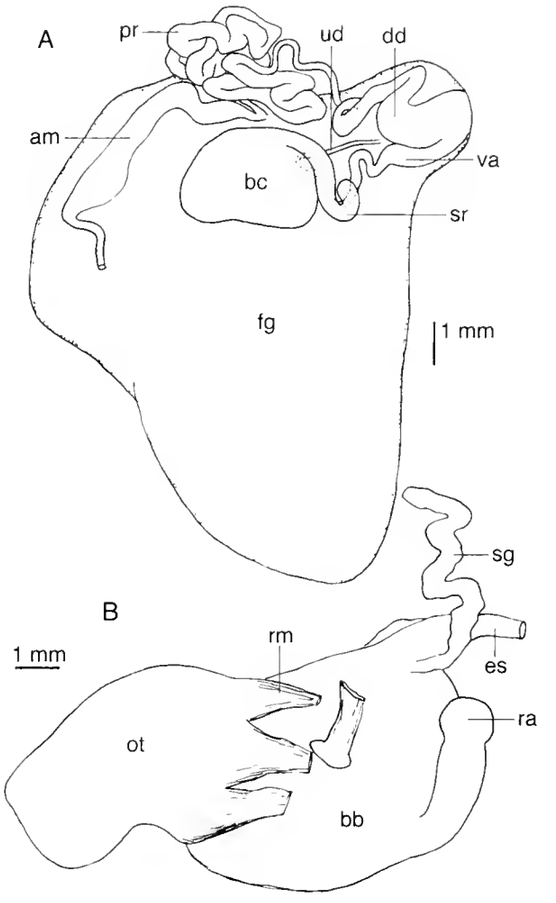

Figure 4. Drawings of the anatomy of *Glossodoris punctilucens* (FSBC I 67201). A, Reproductive system; B, Anterior portion of the digestive system. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; dd, deferent duct; es, esophagus; fg, female gland complex; ot, oral tube; pr, prostate; ra, radular sac; rm, retractor muscle; sg, salivary gland; sr, seminal receptacle; ud, uterine duct; va, vagina.

There are 50 branchial leaves arranged in the typical double spiral of species of *Glossodoris*. The leaves are translucent beige with dark brown spots and opaque white areas. The rhinophores are dark brown to black with bluish-white pigment on the club and a bright orange apex. The sides of the body are dark brown to black with yellow spots. The margin of the foot sole is surrounded by a yellow line.

Reproductive system

The reproductive system contains a long, narrow ampulla that connects to the prostate and the female gland complex (Fig. 4A). The prostate is long and convoluted and narrows into a long, muscular deferent duct that expands into the broad muscular portion of the deferent duct (Fig. 4A). The vagina is long, convoluted and connects directly into the large, irregular bursa copulatrix. The seminal receptacle is very elongate, and also connects directly into the bursa copulatrix next to the vagina insertion. The straight uterine duct leaves

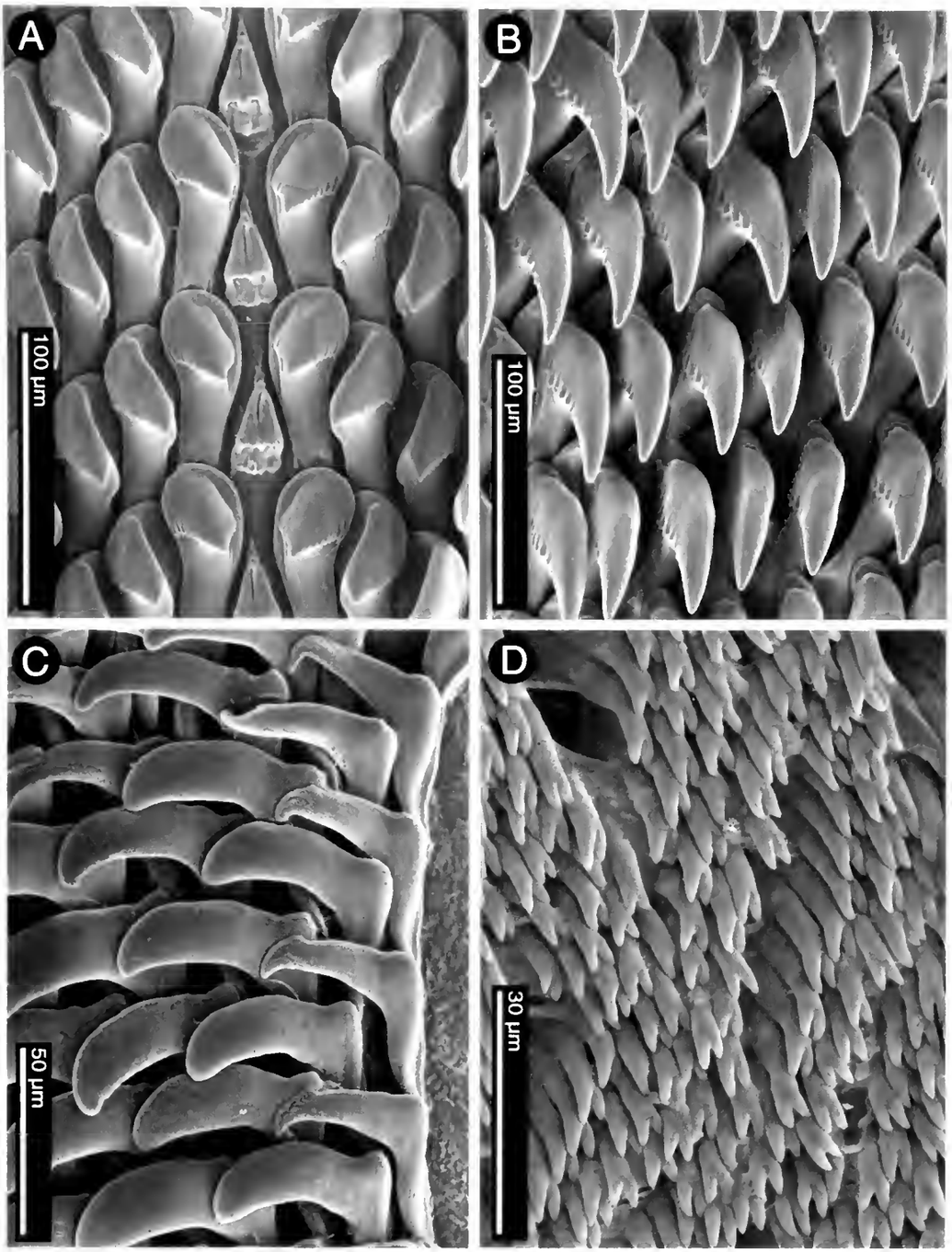

Figure 5. SEM micrographs of the radula and jaws of *Glossodoris punctilucens* (FSBC I 67201). A, Innermost lateral radular teeth; B, Mid-lateral radular teeth; C, Outermost lateral radular teeth; D, Jaw rodlets.

the bursa copulatrix next to the insertion of the seminal receptacle and the vagina, and inserts into the female gland complex, near the opening of the female gland complex.

Digestive system

The buccal bulb is as long as the oral tube (Fig. 4B). The radular formula is 184 × 51.1.51 in the single specimen examined. The rachidian teeth are triangular with a thicker apical region divided by a longitudinal notch. The innermost lateral teeth have a shorter cusp than the rest of the laterals and have 5-7 denticles on both sides of the cusp (Fig. 5A). The mid lateral teeth are hook-shaped with 9-10 denticles on the outer side of the cusp (Fig. 5B). The outer laterals are also hook-shaped and lack denticles (Fig. 5C). The jaw consists of numerous bicuspid (occasionally tricuspid) rodlets (Fig. 5D).

Molecular data

Glossodoris punctilucens is genetically distinct from other species of *Glossodoris* previously studied. The closest species morphologically, *Glossodoris edmundsi* Cervera, García-Gómez, and Ortea, 1989, shows a 7.1% sequence divergence in the COI gene.

Biology

Four of the specimens of *Glossodoris punctilucens* were found on a sponge species tentatively identified as *Ircinia* cf. *campana* (Demospongiae: Dictyoceratida: Irciniidae). Some of the nudibranchs were observed feeding and leaving feeding markings on the sponge, which certainly constitutes their diet. One sponge specimen is deposited at the FWRI collections (FSBC I 069219). *Ircinia campana* belongs to the family Irciniidae (order Dictyoceratida), whereas all known species of *Glossodoris* for which feeding information is available (Rudman and Bergquist 2007) feed on members of the family Thorectidae (order Dictyoceratida). The sponge identification presented here should be regarded as tentative.

Remarks

Chromodoris punctilucens Bergh, 1890 was originally described by Bergh (1890) based on a single specimen collected west of the Dry Tortugas in the Gulf of Mexico at 65 m depth. Subsequently, Valdés *et al.* (2006) published a photograph of another individual collected from an unknown locality in Florida. There are no other published records of this very uncommon species. Subsequent records from the Canary Islands by Odhner (1932) were later identified as a distinct species (see below). Rudman (1984) transferred *C. punctilucens* to *Glossodoris* based on the radular morphology as described by Bergh (1890) and Odhner (1932).

Bergh's (1890) original description and drawings of the radula of *Glossodoris punctilucens* closely match the characteristics of the individual here described. Bergh (1890) described the color of this species as brownish green with numerous yellow and white dots and the mantle margin surrounded by a yellow and a black line. The morphology of the rachidian, inner and outer radular lateral teeth illustrated by Bergh (1890) are virtually identical to those of the specimen here examined.

There are two other Atlantic and one Pacific species similarly colored to *Glossodoris punctilucens*. *Chromodoris ghanensis* Edmunds, 1968 was originally described from Ghana by Edmunds (1968). Edmunds (1968) described this species as having a dark greenish gray or dark bluish gray dorsal color with spots of black, yellow and orange color. The radular morphology is also very similar to that of *G. punctilucens*. Cervera *et al.* (1989) subsequently transferred *C. ghanensis* to *Glossodoris* and described another similar species from the Canary Islands, *Glossodoris edmundsi* Cervera, García-Gómez, and Ortea, 1989 characterized by a grayish-blue dorsum with scattered blackish spots and abundant small orange spots surrounded by smaller yellow ones. Cervera *et al.* (1989) acknowledged the similarities between *G. punctilucens*, *G. ghanensis*, and *G. edmundsi* but distinguished these three species based on anatomical differences, particularly the number of outer radular teeth lacking denticles. Gosliner (1990) re-described specimens of *G. edmundsi* from the Azores and provided the first SEM photographs of the radula, which differs from that of *G. punctilucens* in several details. The rachidian teeth of both Azorean and Madeiran specimens of *G. edmundsi* have a notched, thicker region that occupies almost the entire length of each tooth, whereas in *G. punctilucens* it only occupies the upper 2/3, as observed by Bergh (1890) and the present study. Additionally, the seminal receptacle depicted by Cervera *et al.* (1989) and Gosliner (1990) for *G. edmundsi* is much longer than our observations in *G. punctilucens*. Ortea *et al.* (1996) studied additional specimens from Ghana, Madeira, Azores, and the Canary Islands, and described their reproductive anatomy and radula. Ortea *et al.* (1996) implicitly considered *G. edmundsi* and *G. ghanensis* as synonyms as they included specimens from Ghana, Madeira, Azores, and the Canary Islands under the same species name (*G. edmundsi*), but at the same time discussed several morphological differences between these two species. We were unable to gather enough information from the literature to determine whether *G. edmundsi* and *G. ghanensis* are synonyms, but morphological and molecular evidence presented here suggests that *G. punctilucens* is a distinct species.

Chromodoris dalli Bergh, 1879 was described by Bergh (1879) stating Puget Sound, Washington, USA, as the type locality. The species was later re-described by Bertsch

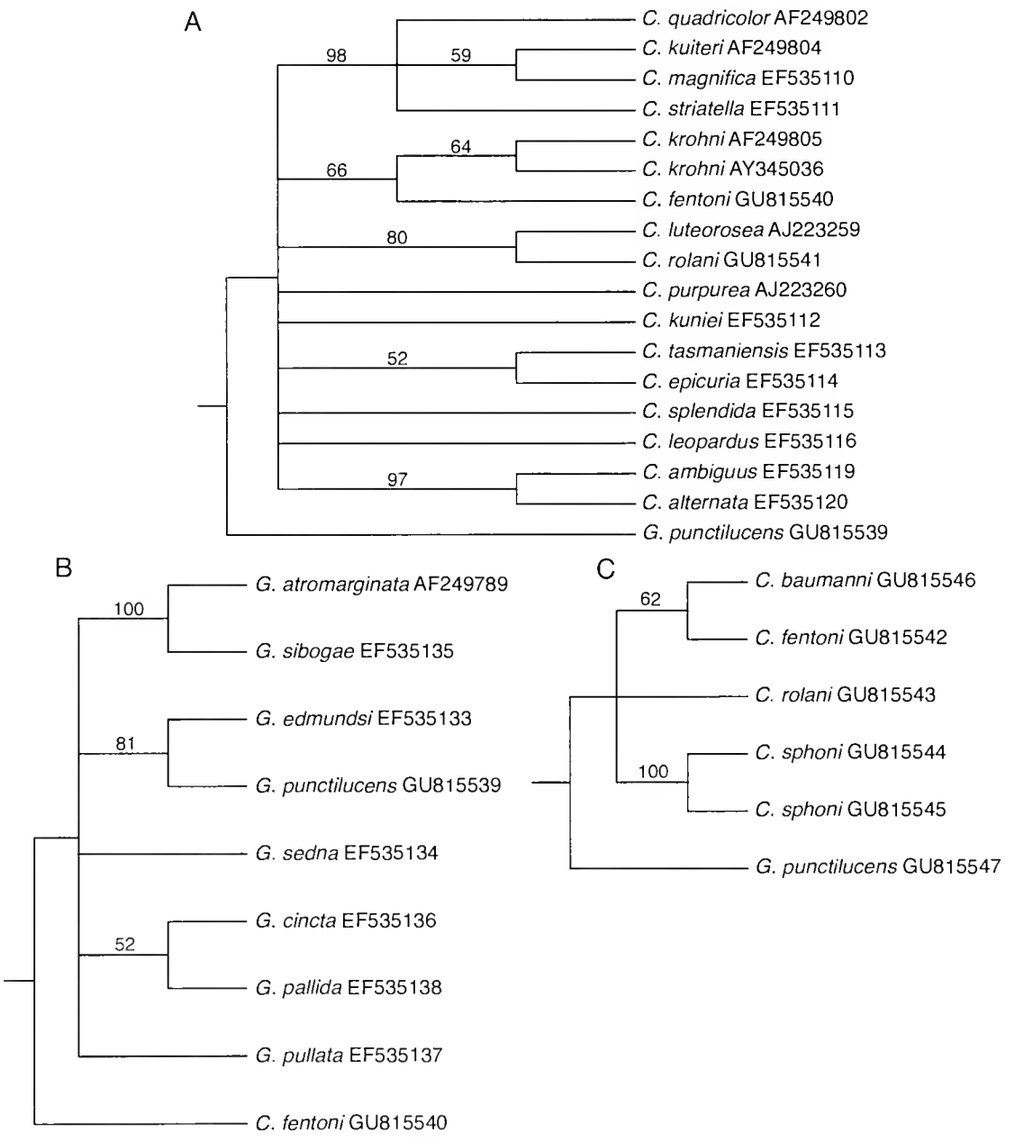

Figure 6. Maximum likelihood bootstrap consensus trees with bootstrap support values. A, COI, *Chromodoris*; B, COI, *Glossodoris*; C, H3, *Chromodoris*. All sequences shown with GenBank accession numbers.

(1978), who suggested that the type material was mislabeled and it was probably collected somewhere in the Gulf of Mexico. Rudman (1984) conclusively transferred this species to *Glossodoris*. *Glossodoris dalli* occurs in the tropical eastern Pacific, from Baja California to the Galápagos Islands (Camacho-García *et al.* 2005). It is characterized by having a lighter color than *G. punctilucens* but both species share numerous tubercles tipped in orange, yellow and white and black spots scattered over the dorsum. The rhinophores and branchial leaves of *G. dalli* are lighter than those of *G. punctilucens* and have orange apices. Ortea *et al.* (1992) described the anatomy and radular morphology of *G. dalli*, which differs from that of *G. punctilucens* in several respects. The seminal receptacle of *G. punctilucens* is curved and inserts directly into the bursa copulatrix, whereas in *G. dalli* it is straight and connects into the vagina, a short distance from the insertion of the vagina into the bursa copulatrix. Ortea *et al.* (1992) described the presence of a vestibular gland in *G. dalli* that was not observed in our specimens of *G. punctilucens*. The radular morphology of *G. dalli* and *G. punctilucens* is very similar, suggesting a close relationship between these two species. Differences between *G. dalli* and *G. edmundsi* include the length of the seminal receptacle, much longer in *G. edmundsi* than in *G. dalli*, and the shape of the rachidian teeth, which have a notched, thicker region that occupies almost the entire length of each tooth in *G. edmundsi*, whereas in *G. dalli*, as in *G. punctilucens*, this thicker region only occupies the upper 2/3 of each tooth.

DISCUSSION

The molecular trees produced in this study (Fig. 6) do not aim to provide a detailed account of the phylogenetic relationships of the two species studied, but just to help, along with the morphological data, to properly characterize the two species. Our goal in providing sequence data along with the description of the new species is to facilitate further work on the systematics and biogeography of *Chromodoris* and test the validity of the new taxon.

For the most part, the COI and H3 bootstrap consensus trees are poorly resolved and most likely would require many more taxa to provide a clear phylogenetic signal; this is however beyond the scope of this paper. The levels of saturation in all the alignments were low and therefore they should not have affected the phylogenetic signal.

Several limited conclusions can be drawn from the phylogenetic trees. The COI tree of the species of *Chromodoris* (Fig. 6A) is poorly resolved and shows very low support for a sister relationship between *Chromodoris fentoni* and *Chromodoris krohni*. However, it is important to note that the sister relationship between two other Atlantic species, *Chromodoris luteorosea* and *Chromodoris rolani*, is well supported. These two very similar species also appear to be distinct. Many other Atlantic and eastern Pacific species need to be included

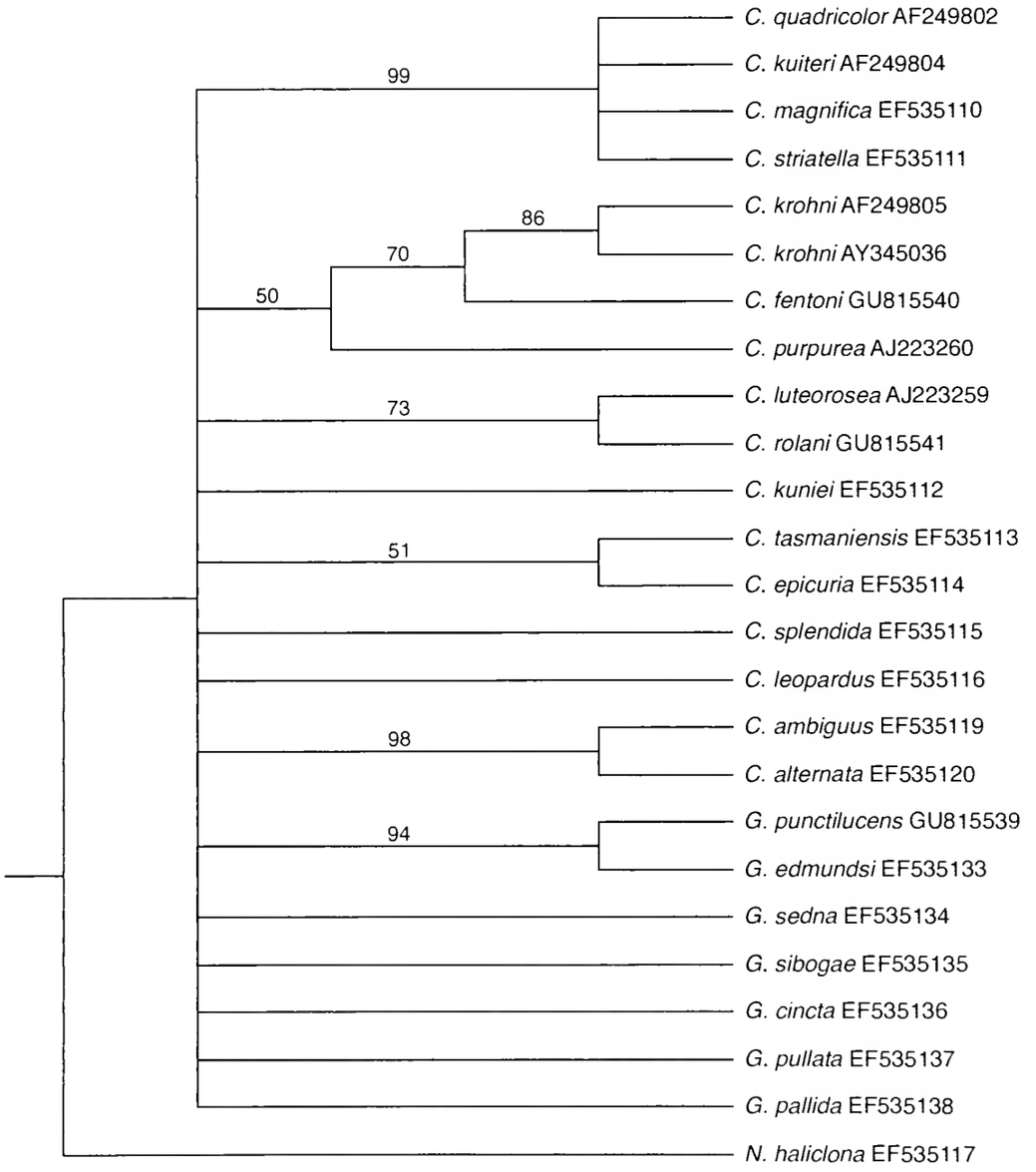

Figure 7. Maximum likelihood bootstrap consensus tree of species of *Chromodoris* and *Glossodoris* with bootstrap support values. All sequences shown with GenBank accession numbers.

in this analysis in order to say anything meaningful about the relationships within this group. Unfortunately we were unable to amplify COI for potentially closely related species such as *Chromodoris sphoni* and *Glossodoris bammani*, from which we obtained H3 sequence data, and we had no access to material from other species such as *Chromodoris luteopunctata*.

The *Glossodoris* COI tree (Fig. 6B) show strong support for the sister relationship between *Glossodoris edmundsi* and *Glossodoris punctilucens*, but as mentioned above, the molecular apomorphies present in these two species as well as anatomical differences justify their separation into two different species.

The combined *Chromodoris* + *Glossodoris* tree (Fig. 7) is also poorly resolved and does not recover the basal relationships between most species and clades. However, this tree provides important information on the phylogenetic position of *Chromodoris fentoni* and *Glossodoris punctilucens*. *Chromodoris fentoni* is nested in a clade containing the two eastern Atlantic species *Chromodoris purpurea* and *C. krohni*, which further supports the placement of *C. fentoni* in *Chromodoris*. Additionally, the combined analysis shows strong support for the sister relationship of *G. punctilucens* and *G. edmundsi*, which appear to be very closely related species. Finally the H3 tree (Fig. 6C) is very incomplete and provides no relevant information except for showing divergence between *Chromodoris fentoni* and other similar species.

The combination of molecular and morphological information in this study provides additional support for the taxonomic decisions. This paper also provides information that will be useful in investigating the relationships among other Caribbean and Gulf of Mexico species of chromodorid nudibranchs.

ACKNOWLEDGMENTS

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Caribbean seagrasses as a food source for the emerald neritid *Smaragdia viridis*

Kimberly K. Holzer[1,2], José L. Rueda[3], and Karen J. McGlathery[1]

[1]Department of Environmental Sciences, University of Virginia, 291 McCormick Road, Charlottesville, Virginia 22904-4123, U.S.A.

[2]Marine Environmental Program, Bermuda Institute of Ocean Sciences, 17 Biological Lane, Saint George's GE01, Bermuda

[3]Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, Málaga 29071, Spain

Correspondence, K. K. Holzer: holzer@virginia.edu

Abstract: Seagrass canopies harbor many different mollusc species, but information about the interaction of these seagrass residents with their host plants remains scarce. Most gastropods inhabiting seagrass meadows are believed to feed on epiphytes rather than directly on living seagrass tissues. In laboratory experiments, we demonstrate that the gastropod *Smaragdia viridis* (Linnaeus, 1758) feeds preferentially on three seagrass species that are common in the Caribbean and Bermuda, including *Thalassia testudinum, Halodule wrightii*, and *Syringodium filiforme*. The percentage of lysed over intact seagrass cells egested was significantly higher in gastropods fed either *T. testudinum* or *H. wrightii* (80.3 ± 4.7% and 84.6 ± 11.4%, mean ± *SD*) but not for *S. filiforme* (59.7 ± 15.9%). Diet versatility for both pioneer (*H. wrightii* and *S. filiforme*) and climax (*T. testudinum*) Caribbean seagrass species allows *S. viridis* to adapt to small-scale disturbances that are common in these habitats, but a diet specializing in seagrass may make this animal vulnerable to large-scale seagrass declines occurring worldwide.

Key words: herbivory, gastropod, *Thalassia*, *Halodule*, *Syringodium*

Seagrass beds represent an important habitat for a large number of mollusc species in the world's coastal oceans (Hemminga and Duarte 2000). Recent data show that herbivory on seagrasses is more important than previously thought, yet the role of small seagrass-feeding invertebrates is still poorly understood (Heck and Valentine 2006). Because most seagrass-associated molluscs (*i.e.*, gastropods) and crustaceans (*i.e.*, amphipods and isopods) feed heavily on epiphytic algae attached to seagrass leaves (Jernakoff *et al.* 1996), small epifauna that also obtain nutrition directly from living seagrass may have a competitive advantage over other mesograzers. Predation on these small seagrass-feeding invertebrates may represent an additional pathway in the mobilization of seagrass carbon from plant material to animals in higher trophic levels, such as crabs and fishes (Castriota *et al.* 2005).

The emerald neritid *Smaragdia viridis* (Linnaeus, 1758) inhabits seagrass beds of the Caribbean Sea, Mediterranean Sea, and probably the western African coasts. This large geographic range seems to be explained by the species' planktotrophic development with larvae remaining in the water column up to 55 days (Scheltema 1971). In the temperate meadows of the Mediterranean Sea, this small gastropod resides in *Cymodocea nodosa* and *Zostera marina* meadows, where leaf epidermal tissues of these plants are its main food source (Rueda and Salas 2007). In these meadows, densities may reach 50 individuals m^{-2}, with maximum values in the summer due to enhanced recruitment (Rueda *et al.* 2009). In the subtropical to tropical meadows of the Caribbean, *S. viridis* has been shown to associate with *Thalassia testudinum* (Scheltema 1971, Lewis and Hollingworth 1982, Thayer *et al.* 1984, Sterrer 1986, Brunt and Davies 1994). The emerald neritid is also found in *Halodule wrightii* and *Syringodium filiforme* pastures (K. Holzer, pers. obs.). The association of *S. viridis* with Caribbean seagrass meadows suggests a possible trophic dependence. In a paper published by Thayer *et al.* (1984: 362), the authors recount,"*S. viridis* roams about the lower half of the green [*Thalassia*] blades and removes a furrow about 1 mm wide and half the thickness of the blade with its radula." A comment on the presence of seagrass epidermal tissues in feces of *S. viridis* was also made in a paper characterizing the epifauna of *Thalassia* meadows in Barbados (Lewis and Hollingworth 1982). However, these two studies did not deal with ingestion of tissues of different seagrass species by *S. viridis*. We addressed this issue in a laboratory experiment where we presented *S. viridis* with tissues of three subtropical to tropical western Atlantic seagrass species (*T. testudinum, H. wrightii*, and *S. filiforme*). Bermuda represents the northernmost limit of these marine angiosperms. We documented the radular marks made on the seagrass leaves and quantified the presence of seagrass cells in the fecal pellets produced during the feeding trial. We also obtained the relative proportion of lysed to intact seagrass cells in the feces as a proxy for digestibility. This study provides data refining the often-cited paradigm that small gastropods in subtropical to tropical seagrass meadows ingest seagrass only incidentally while feeding primarily on algal epiphytes.

MATERIALS AND METHODS

A microcosm feeding experiment was conducted in October 2008 to establish diet preferences of the neritid gastropod *Smaragdia viridis* which is a common component of the epifauna assemblage in seagrass meadows of the Caribbean and Bermuda. In Bermuda we recorded snail densities of 0-150 individuals m^{-2} in different seagrass areas, as well as the presence of the characteristic hemispherical (*ca.* 1 mm diameter) glossy-yellow spawn masses deposited on epiphyte-free seagrass blades at the base of the shoot. For this experiment, neritids were collected by snorkelers on Bermuda's north shore from three shallow (*ca.* 2 m depth) mixed-species beds dominated by *Thalassia testudinum*, and also including *Halodule wrightii* and *Syringodium filiforme*: Fort Saint Catherine (32°23′N, 64°40′W), Higgs Cut (32°22′N, 64°39′W), and Clearwater (32°21′N, 64°39′W). All study animals were found attached to one of the three seagrass species and were never observed on rock or algal substrates. They were acclimated to laboratory conditions for a week and supplied with abundant seagrass-epiphyte forage from their local meadows.

The weeklong feeding trial was performed in an outdoor flowing seawater system exposed to ambient temperature, light, and weather conditions at the Bermuda Institute of Ocean Sciences. Individual microcosms consisted of neutrally buoyant 120 ml transparent plastic containers that were perforated with 14 × 3-4 mm holes to permit constant water exchange. One *Smaragdia viridis* was placed inside each container along with a single-species seagrass diet ($N = 10$). Food items consisted of healthy (green) undamaged 5-10 cm long segments of both new and old seagrass leaves (*Thalassia testudinum, Halodule wrightii*, or *Syringodium filiforme*) and attached epiphytes. Food was collected from Bailey's Bay (32°20′N, 64°43′W) at *ca.* 2 m depth, where epiphyte loads were 1.6 ± 0.5 mg DW cm^{-2} and epiphyte organic content was 25 ± 2% (mean ± *SD*, $N = 10$). Fresh forage was exchanged daily for five consecutive days. On the sixth day, the animals were held in separate glass bowls containing seawater filtered through a 45-µm membrane, and feces were recovered after 24 hours with a pipette.

At the end of each experiment, seagrass blades were examined for radular marks created by the neritid using a binocular microscope, and those areas on leaves containing feeding trails were fixed in Lugol. Feces egested by *Smaragdia viridis* during the experiment were also collected using a pipette and fixed in Lugol for further microscopic analyses. Empty seagrass cells (without cytoplasm and chloroplasts) and intact seagrass cells (with cytoplasm and chloroplasts) were counted in fecal pellets collected during the experiment using the three seagrass species. Components of the epiphyte community (macroalgae, diatoms, and foraminifers) in the feces were also tallied. The potential assimilation of the three seagrass species was then estimated as the percentage of empty seagrass cells compared to the total number of seagrass cells (empty and intact).

Separate Chi-square analyses for each seagrass diet were used to evaluate whether snails (1) prefer seagrass tissues or epiphytes and (2) lyse seagrass cell walls to access the cellular contents. The independent variable in both cases was cell type and the dependent variable was abundance of that cell type in the fecal pellet. Expected values were based on a predicted equal number of (1) seagrass and epiphyte cells and (2) empty and intact seagrass cells, for each of the three seagrass diets. While statistical tests were run on cell abundance, we report the percentage of the different cell types comprising fecal pellets to convey more clearly significant trends in the data.

Smaragdia viridis makes consistent and characteristic grazing trails, independent of season. The weeklong feeding trial was repeated in July 2009 to obtain seagrass and feces samples to photograph.

RESULTS

Smaragdia viridis left radular marks while eating the epidermal tissues of all three seagrass species (Figs. 1A, 1D, 1G). Radular marks were made along the central part of leaves (up to 5 cm long), except for *Syringodium filiforme*, which has a circular leaf cross-section. In *Thalassia testudinum*, the radular marks were less continuous than in both *S. filiforme* and *Halodule wrightii*, and sometimes epidermal tissues of both sides of the *T. testudinum* blade were ingested producing transparent lines (Fig. 1A).

In all cases, fecal pellets were cylindrical (200–400 µm length) with a longitudinal green line (10–20 µm width) rich in chloroplasts. Under microscopic observation, the feces consisted mainly (> 95%) of tissues of the three seagrass species, which were easily distinguished by cell size and shape (Fig. 1C, 1F, 1I). Ungrazed leaves of the three different seagrass species are shown for comparison (Figs. 1B, 1E, 1H). Epiphytes (macroalgae, diatoms, and foraminifers) represented < 3% of the fecal composition and were significantly lower in abundance than seagrass cells (empty and intact) for all seagrass treatments (*Thalassia testudinum* $\chi^2 = 45.9$, $df = 1$, $N = 10$, $P < 0.05$; *Halodule wrightii* $\chi^2 = 15.1$, $df = 1$, $N = 10$, $P < 0.001$; *Syringodium filiforme* $\chi^2 = 23.0$, $df = 1$, $N = 10$, $P < 0.001$) (Fig. 2A). The abundance of empty seagrass cells was significantly higher than intact cells for *Thalassia testudinum* ($\chi^2 = 18.2$, $df = 1$, $N = 10$, $P < 0.001$) and *Halodule wrightii* ($\chi^2 = 9.4$, $df = 1$, $N = 10$, $P < 0.01$), but not for *Syringodium filiforme* ($\chi^2 = 3.3$, $df = 1$, $N = 10$, $P > 0.05$) (Fig. 2B).

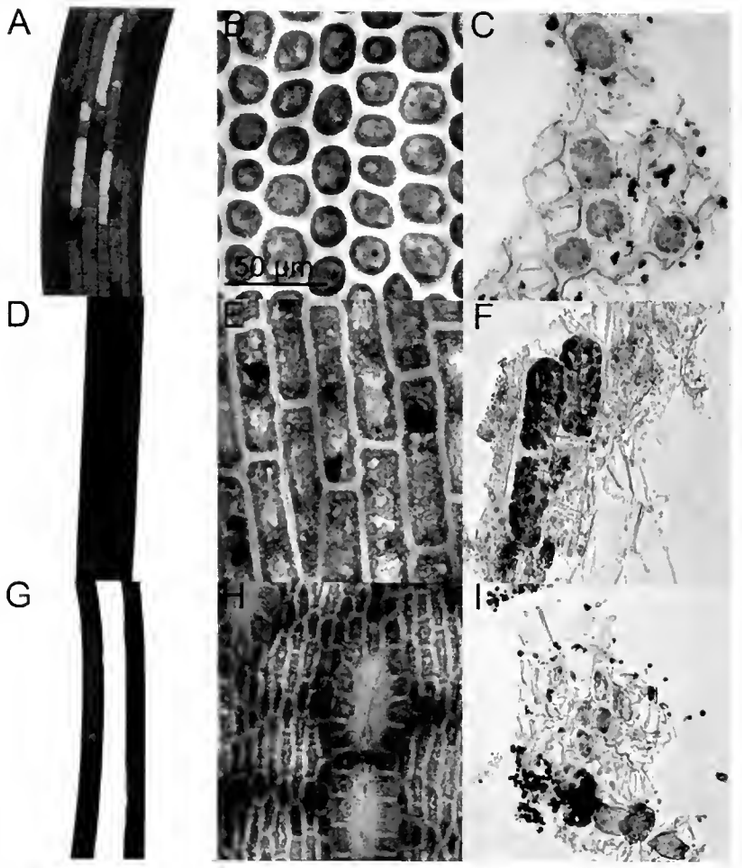

Figure 1. Radular marks (A, D, G) and feces (C, F, I) of *Smaragdia viridis* after grazing *Thalassia testudinum* (A–C), *Halodule wrightii* (D-F), and *Syringodium filiforme* (G-I). Unaltered cells of leaf tissues from *T. testudinum* (B), *H. wrightii* (E), and *S. filiforme* (H) are shown for comparison. Scale bar is the same for feces and unaltered cells of seagrass leaf tissues. *Thalassia testudinum* leaf width: 7.1 mm; *H. wrightii* leaf width: 1.8 mm; *S. filiforme* leaf width: 0.8 and 0.9 mm.

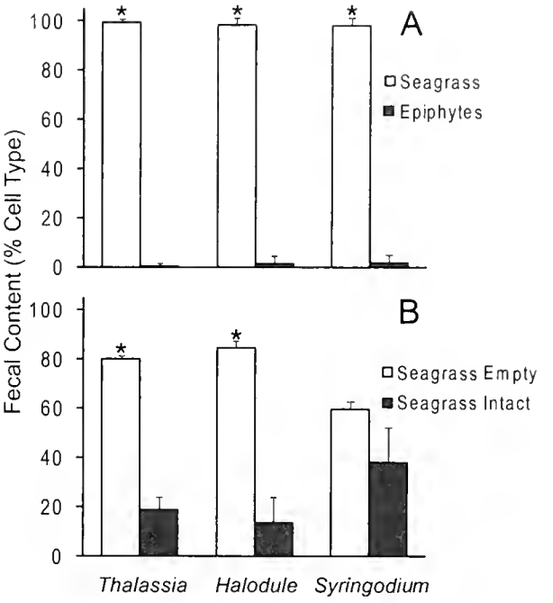

Figure 2. Mean (+ *SD*) fecal content of *Smaragdia viridis* after feeding on three different seagrass species. (A) Percentages of seagrass (empty and intact) and epiphyte (macroalgae, diatoms, and foraminifers) cells in snail feces (N = 10). (B) Percentages of empty (without cytoplasm) and intact (with cytoplasm) seagrass cells in snail feces (N = 10).

DISCUSSION

We present experimental data that *Smaragdia viridis* feeds on the Caribbean seagrass species *Thalassia testudinum*, which confirm that this gastropod directly ingests seagrass tissue and support the brief anecdotal observations in Thayer *et al.* (1984) and Lewis and Hollingworth (1982). Our laboratory data show that this neritid also feeds on at least two other Caribbean seagrasses, including *Halodule wrightii* and *Syringodium filiforme*, Our results support an emerging trend of direct feeding on seagrass by other *Smaragdia* species. Seagrass-dependence has been documented for *S. bryanae* (Pilsbry, 1917) with *H. hawaiiana* in Hawaii by Unabia (1980) and for *S. souverbiana* (Montrouzier, 1863) with *H. ovalis* and *H. spinulosa* in eastern Australia (J. Rueda, pers. obs.). In the Mediterranean Sea, *S. viridis* is considered a different subspecies (*S. viridis viridis* Linnaeus, 1758) than in the Caribbean Sea and it also feeds on seagrasses, but of different genus and species, such as *Zostera marina* and *Cymodocea nodosa* (Rueda and Salas 2007). Other *Smaragdia* species also strictly associate with seagrasses, such as *S. rangiana* (Récluz, 1841) with *Thalassia hemprichii* in the Republic of Seychelles (Taylor and Lewis 1970), but information on feeding is presently unavailable. There are also less common *Smaragdia* species, such as *S. tragena* (Iredale, 1936) or *S. roseopicta* (Thiele, 1930), for which habitat preference is undocumented (Loch 1994), and more work is clearly needed to confirm that all species in the *Smaragdia* genus associate with and feed on seagrasses.

We also demonstrate that *Smaragdia viridis* grazing can rupture plant cell walls, providing access to the more nutritious cell contents. Previous studies in marine and terrestrial ecosystems suggest that grasses coevolved with herbivores by defending themselves through producing high silica and fiber levels in their cell walls, and that some herbivores (*e.g.*, mammals, fishes) have evolved special dentition to accommodate plant toughness (review, Thayer *et al.* 1984). Our data indicate that *S. viridis* uses a specialized feeding technique to break apart plant cell walls and empty the more palatable cell content inside. By using the radula to destroy the physical integrity of the cells, the snails can gain access to the cytoplasm without necessarily having to digest the cellulose of the cell wall or rely on symbiotic gut flora. It is possible that other small invertebrates also use the same process to destroy cell integrity and ingest the cytoplasm, and that this is a mechanism by which substantial energy enters higher trophic levels.

While some of the palatable cytoplasm may leak into the environment during feeding, evidence that *Smaragdia viridis* assimilates living seagrass tissues comes from isotopic data collected in August 2008 showing that emerald neritid tissues contain $\delta\ ^{13}C$ values (-8 to -9 ‰) close to those of green *Thalassia* leaves but significantly more enriched than the epiphyte-periphyton consortium attached to the leaves (K. Holzer, unpubl. data). Seasonal variability in epiphyte loads and epiphyte composition are not expected to alter the contribution of seagrass to the diet of *S. viridis* since field observations indicate that the emerald neritid targets emergent epiphyte-free leaf tissue at the base of shoots (Thayer *et al.* 1984, Rueda *et al.* 2009, K. Holzer, pers. obs.).

Information on ingestion of food is limited in marine grazing gastropods compared to other molluscs (*e.g.*, filter feeding bivalves) (Saleuddin and Wilbur 1983), but absorption of ingested food has been positively correlated with the quality of the food in bivalves (Iglesias *et al.* 1996, Hawkins *et al.* 1998). Our data indicated that the proportion of broken over intact seagrass cells egested was higher only for *Thalassia testudinum* and *Halodule wrightii*-fed snails and not for *Syringodium filiforme* (Fig. 2B). A different pattern was found for *S. viridis* feeding on *Zostera marina* and *Cymodocea nodosa*, with lower percentages of broken cells for the later (J. Rueda, unpubl. data). This relationship may be linked to a higher amount of cell wall material in relation to cell cytoplasm in *C. nodosa* compared to *Z. marina*. Fittingly, the three tropical Caribbean seagrasses contained different cell sizes with the largest cells for *H. wrightii* followed by *T. testudinum* and the smallest cells for *S. filiforme* (Fig. 1B, 1E, 1H). A large number of small cells may result in compact tissue that is more difficult to digest than tissues consisting of much larger cells with a lower combined surface area and lower proportion of lignified cell wall material. Alternatively, leaves of *S. filiforme* are rounded not flattened like the other two seagrasses, which could impede the grazing process.

In summary, *Smaragdia viridis* shows diet flexibility among seagrass species and can consume the pioneer Caribbean seagrasses *Halodule wrightii* and *Syringodium filiforme* as well as the climax species *Thalassia testudinum*. Grazing along the succession sequence of seagrass makes *S. viridis* suited to recovery following small local disturbances. However, this seagrass-dependent snail may also be susceptible to accelerating trends of global seagrass decline (*i.e.*, Waycott *et al.* 2009). Conversely, the formation of lesions on live seagrass blades from the rasping activity of these snails (Fig. 1A, 1D, 1G) may suppress plant growth as has been shown for the periwinkle *Littoraria irrorata* (Say, 1822) on salt marsh grass (Silliman and Zieman 2001) or the limpet *Tectura depicta* (Hinds, 1842) on eelgrass (Zimmerman *et al.* 1996). The radula of *S. viridis* may also puncture lacunal air channels that transport gases from seagrass leaves to roots, causing leakage (Fig. 3) and an interruption of oxygen delivery to the rhizosphere. We suggest that a reduction of predatory crabs or fishes, through overfishing, could increase the abundance of *S. viridis* and exacerbate the potentially harmful impacts of neritid feeding on the seagrass host.

Figure 3. *Smaragdia viridis* actively feeding on a *Halodule wrightii* shoot. Arrows identify bubbles leaking from the new grazing-lesion.

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Survival and growth of newly transformed *Lampsilis cardium* and *Lampsilis siliquoidea* in a flow-through, continuous feeding test system

Jeffery R. Meinertz, Theresa M. Schreier, Karina R. Hess, and Michelle R. Bartsch

U.S. Geological Survey, Upper Midwest Environmental Sciences Center, Biological Resources Division, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603, U.S.A.

Correspondence, J. R. Meinertz: jmeinertz@usgs.gov

Abstract: A test system was evaluated for assessing chronic toxicity of waterborne chemicals with early life stage mussels. To determine if the test system could result in ≥80% survival in a control (unexposed) group, fat mucket mussels (*Lampsilis siliquoidea* Barnes, 1823) and plain pocketbook mussels (*L. cardium* Rafinesque, 1820) 1 day post transformation were stocked into test chambers (250 mL beakers, water volume, 200 mL, 21 °C, 40 mussels of 1 species per chamber) within a test system constructed for conducting chronic, continuous exposure, flow-through toxicity tests. The test system contained 60 chambers containing silica sand, 30 chambers with *L. siliquoidea*, and 30 with *L. cardium*. Each chamber in the continuous feeding system received 1 of 6 food types prepared with concentrated algal products. After 28 days, mussels were harvested from chambers to assess survival and growth. For *L. siliquoidea*, mean survival ranged from 34 to 80% and mean shell length ranged from 464 to 643 µm. For *L. cardium*, mean survival ranged from 12 to 66% and mean shell length ranged from 437 to 612 µm. The maximum mean growth rate for *L. siliquoidea* was 12.7 µm/d and for *L. cardium* was 11.8 µm/d. When offered a continuous diet of *Nannochloropsis*, *Tetraselmis*, and *Chlorella* for 28 days in the test system, the survival of 1 day post transformation *L. siliquoidea* was 80%. The test system can be easily enhanced with a pumping system continuously delivering test chemical to the test system's flow stream allowing for chronic toxicity tests with 1 day post transformation mussels.

Key words: juvenile mussels, laboratory cultures, diet, chronic exposure

Freshwater mussels are some of the most imperiled organisms in North America. Of the nearly 300 taxa of freshwater mussel populations in North America, 70 species (23%) are listed as endangered or threatened and another 40 species (14%) are candidates for listing as endangered or threatened (Williams *et al.* 1993, Neves 1997, 2004, Graf and Cummings 2007). The causes for the declines in unionid abundance and diversity have not been well characterized. Potential anthropogenic stressors that may be contributing to the declines include siltation, dams, mining wastes, introduction of exotic bivalve species such as zebra mussels (*Dreissena polymorpha* Pallas, 1771) and Asian clams (*Corbicula fluminea* Müller, 1774), industrial wastes, agricultural point and nonpoint pollution, and pharmaceuticals and personal care products.

Recent publications have reported the sensitivity of freshwater mussels to contaminants including pesticides, ammonia, chlorine, and heavy metals (Valenti *et al.* 2005, 2006, Bringolf *et al.* 2007a, 2007b, 2007c, Wang *et al.* 2007a, 2007b). Much of the work was conducted in accordance with the American Society for Testing and Materials (ASTM 2006) Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels. The ASTM guide recommends that acute toxicity tests (<14 days) be initiated with juvenile mussels within 5 days post transformation and that chronic studies (>14 days) be conducted with mussels 60 to 120 days post transformation. The age designation is based on high mortality of newly transformed mussels cultured for more than 7 days in laboratory settings (Gatenby *et al.* 1996, 1997, Jones *et al.* 2004). Mortality after 7 or 14 days has been attributed to an inappropriate food supply and not understanding of nutritional requirements during this life stage (Ingersoll *et al.* 2007). The conventional wisdom is that newly transformed juveniles depend on pedal feeding to obtain food, presumably using cilia on the foot to move food. Later in development, it is thought that juveniles begin to use a combination of pedal and suspension feeding, with more emphasis on suspension feeding with increased age.

The ASTM guide indicates that for acute trials (≤4 days) initiated with juvenile mussels <5 days post transformation, survival in the control groups is typically >90%. For chronic trials (10-28 days) with mussels 2 to 4 months post transformation, survival in the control groups is typically >80%. These recommendations are based on mortality during the apparent sensitive stage from 14 to 42 days post transformation. Although mussels live in sediment, the ASTM guide recommends that mussel toxicity trials be conducted with water only, presumably eliminating sediment as a potentially complicating factor in the trial.

It is generally understood that the earliest life stages of aquatic organisms are the most sensitive. As for mussels, Valenti *et al.* (2006) found that mussel sensitivity to chlorine

decreased as mussel age increased. Initiating chronic studies with 2 to 4 months post transformation mussels potentially overlooks critical life stages beginning immediately after transformation through the next 2 months. This study evaluated a test system for suitability to assess the chronic toxicity of waterborne chemicals to mussels <2 months of age. The test system was evaluated by determining survival and growth of 1 day post transformation *Lampsilis cardium* (Rafinesque, 1820) and *Lampsilis siliquoidea* (Barnes, 1823), after 28 days of culture with 1 of 6 food sources. This test system will be beneficial by generating chronic toxicity data necessary to ensure that water quality criteria for individual chemicals are set or revised to protect all aquatic organisms, including early life stage mussels.

MATERIAL AND METHODS

Experimental animals

Largemouth bass (*Micropterus salmoides*; $N = 48$; length, 8-13 cm) were equally distributed to 12 flow-through 38-L aquaria. Five days later, *Lampsilis cardium* glochidia were removed from adult mussels (acquired from Pool 7 of the Mississippi River, Wisconsin) by prying open the shells and flushing glochidia out with a stream of well water from a 10-mL syringe. Glochidial viability was verified by the rapid closure of shells when glochidia in a subsample were exposed to a sodium chloride solution (ASTN 2006). All glochidia were mixed in 1 common container. Twenty four largemouth bass were infested with glochidia by transferring groups of 4 fish to about 2 L of aerated well water containing an aliquot of the glochidial slurry. About 5 min later, fish were returned to their respective aquaria. The same procedures were repeated with adult *Lampsilis siliquoidea* mussels.

Exposure system

The system was constructed with materials and operated with parameters described in the standard guide for conducting toxicity trials with freshwater mussels (ASTM 2006). The test system was set up in an enclosed chamber where incandescent lighting provided 16 hours of light and 8 hours of darkness with 20 minute transition periods for lights to fade on and off. Test chambers ($N = 60$) were supported on platforms elevated over a fiberglass tank. Test chambers were 250-mL glass beakers with a 12-mm hole drilled in the bottom of each beaker. A hole was drilled through silicone stoppers and glass standpipes inserted into each stopper which were inserted into the beaker holes. The height of standpipes was set for a water volume of 200 mL. Nitex® bolting cloth (161 µm mesh; mention of trade or manufacturer name is solely for providing specific information and does not imply endorsement by the U.S. Geological Survey) covered standpipe openings inside the beakers. Stoppers, standpipes, and bolting cloth were secured with silicone sealant. Silica sand (blend FW 120, Badger Mining Corporation, Berlin, Wisconsin) was sieved to a common size range (75 to 150 µm) using a Retsch® sieve shaker (F. Kurt Retsch GmbH & Co., Germany) and USA standard testing sieves (Soiltest Inc., Evanston, Illinois). Silica sand (25 ± 1 g) was dispensed into each test chamber.

Distribution boxes (1 box for each food type) delivered food solutions to the test chambers through polyethylene tubing (PE160), and were mounted above the test chambers. A head box was mounted above the distribution boxes. Flow of well water from the head box to distribution boxes (186-201 mL/min) was controlled by a 3-way polycarbonate stopcock (1 for each distribution box). Food solutions entered their respective flow streams above the distribution boxes through a second polycarbonate stopcock. The nominal flow rate from distribution boxes to the test chambers was 10 mL/min.

Food preparation and food delivery

All food types were prepared with Reed Mariculture (Campbell, California) concentrated products and did not contain live organisms. Each food type was prepared daily in 2-L volumetric flasks. After adding the prescribed volumes of concentrated products, flasks were filled with about 250 mL of well water, swirled, and allowed to stand for about 1 min to prevent cytolysis before the process was repeated at least 4 more times. Thereafter, the flask volumes were adjusted to 2 L with well water.

Food type 1 was prepared with 2.3 mL of *Nannochloropsis* 3600 Instant Algae® (a marine green microalga). Food type 2 was prepared with 1.2 mL of *Nannochloropsis* 3600 Instant Algae and 1.2 mL of *Tetraselmis* (a marine large green flagellate). Food type 3 was prepared with 1.2 mL of *Nannochloropsis* 3600 Instant Algae, 0.6 mL of *Tetraselmis*, and 0.8 mL of marine *Chlorella* (a marine green microalga). Food type 4 was prepared with 1.2 mL of *Nannochloropsis* 3600 Instant Algae and 2.8 mL of *Thalassiorsira weissflogii* (a large marine diatom). Food type 5 was prepared with 1.2 mL of *Nannochloropsis* 3600 Instant Algae and 2.4 mL of *Pavlova* (a small golden brown flagellate). Food type 6 was prepared with 1.2 mL of *Nannochloropsis* 3600 Instant Algae, 1.6 mL of *T. weissflogii*, and 1.2 mL of *Pavlova*. At about 2 weeks, a layer of organic material accumulated on top of the sand in most test chambers. Ingredients were reduced by one half throughout the remaining 2 weeks of the study.

Food solutions were continuously mixed on stir plates while being delivered (1 mL/min) to the test system through Masterflex® Tygon® L/S 13 LFL (through the pump head) and Masterflex C-Flex® L/S 13 (spliced to the LFL tubing after the pump head and connected to the polycarbonate

stopcock above the distribution box) tubing using a Masterflex® Digi Staltic pumping system (Cole-Parmer, Vernon Hills, Illinois). Flows of food solutions through the test system were initiated 1 day before mussels were stocked into test chambers.

Collection and distribution of juveniles

Each day during the infestation period, the bottoms of aquaria were siphoned to remove detritus and newly transformed mussels. Sixteen days after infesting fish with *Lampsilis siliquoidea* glochidia, newly transformed *L. siliquoidea* were collected by filtering siphoned water through a 153-μm sieve made with Nitex bolting cloth. The following day, groups of 20 mussels displaying foot movement were randomly stocked into each of 30 chambers until each chamber had 40 mussels. Concurrent with stocking the test chambers, 1 day post transformation mussels ($N = 16$) with foot movement were stored in a glass vial filled with 10% buffered formalin for 1 day, then transferred to 70% ethanol.

Newly transformed *Lampsilis cardium* were collected 17 days after infesting fish with *L. cardium* glochidia. The following day, 30 test chambers were stocked according to the procedures previously described and an additional 22 1-day post transformation mussels were stored in 10% buffered formalin, then 1 day later, transferred to 70% ethanol.

General husbandry

Each day, water temperature, dissolved oxygen, and pH were measured and water flow to each chamber was verified or measured. Three times during the study, light intensity was measured directly over the chambers. Once per week, water alkalinity and hardness were measured in water samples taken from the head box.

Mussel collection and shell length measurement

Twenty eight days after stocking each species, the contents of each chamber were sieved through a 202 μm sieve. The material collected on the sieve was rinsed into a petri dish. Live and dead mussels were enumerated with the aid of a dissecting microscope. Mussels were considered alive if foot movement or ciliary activity was observed. Live mussels from each chamber were enumerated, transferred to 10% buffered formalin for 1 day then transferred to 70% ethanol.

Shell length (the maximum distance across the shell parallel to the hinge) was measured from a digital photomicrograph of the mussel (QImaging Micropublisher 3.3 RTV digital camera, QImaging, Canada; Nikon Eclipse model E600 microscope, Nikon Corporation, Melville, New York). Shell length was measured using image analysis software (Image Pro® Express, ver. 6.0.0.319, Media Cybernetics, Silver Spring, Maryland) that was calibrated from a photomicrograph of a stage micrometer.

Data analyses

Each food type was delivered to 5 test chambers with *Lampsilis cardium* and 5 chambers with *L. siliquoidea*. Each chamber was randomly assigned to 1 of 10 blocks so that each food type was represented in each block (a randomized block design in a 2×3 configuration). Feed type 1 was labeled as the control diet for statistical comparisons. Each side of the test system (30 chambers on each side) was assigned to 1 of the 2 species.

Percent survival was determined by comparing the number of live mussels found with the number of mussels stocked into each chamber. Growth rate was determined by subtracting the mean shell length of the 1 day post transformation mussels from the shell length of mussels collected at the end of the trial. The difference was divided by the trial duration (28 days) to calculate a daily growth rate.

The mussels could experience one of two outcomes, survival or death. Survival and death are therefore binary random variables that presumably follow Bernoulli distributions. The response data may be categorized as either the numbers of survivors or the numbers of dead, out of a fixed number of mussels in each test chamber. The numbers of survivors or dead, therefore, follow a binomial distribution. In this analysis, the focus was on the proportional risk of survival versus death. Test chambers were the fundamental experimental units. Mussels held in the same chamber experienced identical environmental conditions but those conditions may have varied randomly among chambers within the same treatment group. Mussels were continuously offered various food solutions for 28 days. Therefore, food solution was considered a categorical (classification) variable rather than a continuous variable in the data analysis because each food solution contained a different algal species or combination. The probability of death at day 28 was modeled by a generalized linear mixed model fitted using SAS GLIMMIX (Wolfinger and O'Connell 1993). The correlation between the two types of outcomes for the test chamber population was modeled with shared (G-side) random effects with food solution assignment as a grouping variable. Over dispersion within the model was modeled using an R-side covariance structure. Determination of significant differences between feed types was accomplished by comparison of least-square means.

Shell length (μm) and growth rate (μm/day) were determined only for mussels that survived until the end of the study. Data were evaluated to ensure assumptions of normality (Shapiro and Wilk 1965). Shell length and growth rate data as a function of food solution were fit to a generalized linear model (McCullagh and Nelder 1989) using SAS GLM, and model parameters and 95% confidence intervals were estimated using maximum-likelihood estimation. Likelihood-ratio F-tests were used to test hypotheses about the relation

between shell length or growth rate and food solution. Determination of significant differences between feed types was accomplished by pair-wise comparison of least-square means using a Bonferroni adjustment for multiple comparisons. All statistical analyses were performed with SAS software (SAS 2003) and analyses considered to be significant if $P \leq 0.05$.

RESULTS

Physical parameters of the test system

The mean water temperature throughout the trial was 20.8 °C. Mean dissolved oxygen concentrations for each food type for each species ranged from 7.77 to 7.96 mg/L. Mean pH for each food type for each species ranged from 7.32 to 7.34. Mean alkalinity was 107 mg/L as $CaCO_3$ and mean hardness was 167 mg/L. Light intensity measurements over the test chambers ranged from 40 to 160 lux. Mean flow rate through test chambers was 9.9 mL/min with individual measurements ranging from 87.0 to 10.8 mL/min.

Recovery of mussels from test chambers and mussel survival

The mean recovery of live and dead *Lampsilis siliquoidea* from each test chambers was >87% (Table 1). Survival of *L. siliquoidea* ranged from 33.5 to 80.0% among all food types (Table 1). Food type explained a significant amount of the variation in *L. siliquoidea* survival (Chi-square test, $\chi^2 = 8.40$, $df = 5$, $P < 0.01$). Food type 3 resulted in the greatest percent survival although survival resulting from food types 1 and 4 were not statistically different. Survival variability with those food types were 13, 20, and 8.7% relative standard deviation (RSD), respectively. Comparing food type 3 and 4 (each with similar survival and variability ≤ 20% RSD), mussels fed food type 3 were 1.3 times more likely to survive than if fed food type 4.

The mean recovery of live and dead *Lampsilis cardium* from each test chamber was >83% (Table 1). Survival for *Lampsilis cardium* ranged from 12.0 to 66.0% among all food types (Table 1). Food type explained a significant amount of the variation in *L. cardium* survival ($\chi^2 = 6.06$, $df = 5$, $P < 0.01$). Food type 1 resulted in the greatest percent survival although survival generated by food types 2, 3, and 4 were not statistically different. Only food type 3 with a survival of 60.5% had a variability value of <20% RSD.

Mussel growth

The mean shell length of 1 day post transformation *Lampsilis siliquoidea* was 287 µm (5.4% RSD). *Lampsilis siliquoidea* mean shell lengths after 28 days ranged from 464 to 643 µm among all the food types (Table 2). Food type explained a significant amount of the variation in *L. siliquoidea* growth rate ($\chi^2 = 4{,}517$, $df = 5$, $P < 0.01$). Shell lengths of *L. siliquoidea* offered food type 3 were significantly greater than shell lengths resulting from any other food type. Growth rates for *L. siliquoidea* ranged from 6.3 to 12.7 µm/d and were significantly greater in mussels offered food type 3 than in mussels offered any other food type (Table 2).

The mean shell length of 1-day post transformation *Lampsilis cardium* was 283 µm (5.9% RSD). Mean shell lengths of *L. cardium* after 28 days ranged from 437 to 612 µm among all the food types (Table 2). Food type explained a

Table 1. Recovery and survival of *Lampsilis siliquoidea* and *Lampsilis cardium* after stocking the test system with mussels 1 day post transformation and offering mussels 6 feed types continuously for 28 days. Each species was represented with 30 chambers, 40 mussels per chamber, each chamber receiving 1 of 6 feed types. Mean values of the same species denoted with a common letter are not significantly different. *SD*, standard deviation; RSD, relative standard deviation.

Species	Food type	Mean recovery (%)	*SD* (%)	RSD (%)	Mean survival (%)	*SD* (%)	RSD (%)
Lampsilis siliquoidea	1	83.0	14	17	67.1[a,b]	13	20
	2	83.5	16	20	56.5[a,c]	19	34
	3	88.0	11	13	80.0[b]	11	13
	4	91.0	8.9	9.8	74.8[b]	6.5	8.7
	5	88.5	4.2	4.7	42.5[a,c]	27	63
	6	89.5	12	13	33.5[c]	12	35
Lampsilis cardium	1	84.5	14	16	66.0[a]	16	23
	2	84.0	13	16	43.4[a,c]	22	50
	3	79.0	8.0	10	60.5[a]	6.5	11
	4	84.0	7.0	8.3	51.0[a]	26	52
	5	81.0	15	18	12.0[b]	12	99
	6	89.0	5.2	5.8	23.5[b,c]	9.8	42

Table 2. Shell lengths and growth rates of *Lampsilis siliquoidea* (mean shell length at 1 day post transformation, 287 μm) and *Lampsilis cardium* (mean shell length at 1 day post transformation, 283 μm) after stocking the test system with mussels 1 day post transformation and offering mussels 6 feed types continuously for 28 days. Each species was represented with 30 chambers, 40 mussels per chamber, each chamber receiving 1 of 6 feed types. Means of the same species denoted with a common letter are not significantly different. *SD*, standard deviation; RSD, relative standard deviation.

Species	Food type	*N*	Mean shell length (μm)	*SD* (μm)	RSD (%)	Min (μm)	Max (μm)	Mean growth rate (μm/d)
Lampsilis siliquoidea	1	124	495[a,d]	79	16	334	697	7.4
	2	104	464[b]	66	14	323	642	6.3
	3	152	643[c]	102	16	335	870	12.7
	4	144	509[a]	70	14	360	681	7.9
	5	35	471[b,d]	63	13	360	595	6.6
	6	55	485[a,b]	70	14	344	623	7.1
Lampsilis cardium	1	109	512[a]	75	15	278	676	8.2
	2	73	437[b]	49	11	350	596	5.5
	3	102	612[c]	86	14	395	785	11.8
	4	91	535[d]	57	11	386	628	9.0
	5	20	510[a,d,e]	70	14	372	628	8.1
	6	43	476[e]	62	13	329	616	6.9

significant amount of the variation in *L. cardium* growth rate (χ^2 = 4,240, *df* =5, $P < 0.01$). Shell lengths of *L. cardium* offered food type 3 were significantly greater than shell lengths resulting from any other food type. Growth rates for *L. cardium* ranged from 5.5 to 11.8 μm/d and were significantly greater in those mussels offered food type 3 than in mussels offered any other food type (Table 2).

DISCUSSION

After 28 days in the test system, *Lampsilis siliquoidea* proved to be more rugged (max. survival, 80%) than *Lampsilis cardium* (max. survival, 66%). Statistically, *L. siliquoidea* specimens were about 2.3 times more likely to survive than *L. cardium*. Both species responded similarly to any particular food type: *i.e.*, if one species responded poorly to a particular food type, the other species did as well.

Growth rates attained in this study (*Lampsilis siliquoidea*, 12.7 μm/d; *Lampsilis cardium*, 11.8 μm/d) were consistent with growth rates previously reported. Barnhart (2006) reported lampsiline growth rates ranging from 4.7 to 12.2 μm/d in a recirculating laboratory system with cultured algae as a food source. Gatenby *et al.* (1997) measured growth rates of 3.9 to 10.4 μm/d through a 140-day period for *Villosa iris* (I. Lea, 1830) held in aerated dishes and fed various diet combinations of cultured algae and silt. Juvenile *Lampsilis fasciola* (Rafinesque, 1820) had growth rates of about 15 μm/d through a 105 or 112-day period when held in a recirculating system and fed cultured algae (Steg 1998). Newton *et al.* (2003) also reported *L. cardium* growth rates ranging from 4.2 to 7.2 μm/d for laboratory reared mussels. Newton *et al.* (2003) measured growth rates after mussels (3 to 5 days post transformation) were held in well water at 22 °C with no food source for periods of 4 and 10 days.

In our test system, feed type significantly affected shell length and growth rate for each species. As with survival, shell length and growth rate response of each species was similar for a given food.

The recovery of live and dead mussels from individual test chambers of each species ranged from 60 to 100%. Several factors may have contributed to mussel recoveries less than 100%. First, mussels may have been compromised during the stocking process when handled with a pipette at least twice and agitated several times with swirling and rinsing. If mussels were damaged to the point of causing early mortality, then the shells from those dead mussels may not have been discernable at the end of the study for enumeration. The shells from newly transformed mussels are relatively fragile and may have either deteriorated through time or disintegrated during reconnaissance procedures at the end of the study.

There were a few cases where mussel recovery was >100%. A plausible reason for the errant recovery may have been the counting of 1 shell from a recently dead mussel as 1 whole mussel. The orientation of the shell in the petri dish may not have allowed for discerning whether the shell was a whole mussel or only 1 of 2 shells separated during the recovery procedures. When more than 40 mussels were recovered, adjustments were made in the recovery and survival calculations. Although considered to be a conservative approach, this method of calculating recovery and survival did not substantially affect the data.

Most systems for rearing newly transformed mussels in laboratory settings have been designed for propagation rather than for conducting chronic toxicity tests (Gatenby *et al.* 1996, 1997, Jones and Neves 2002, Jones *et al.* 2004, Barhhart 2006, Kovitvadhi *et al.* 2006, 2008). Our test system was instead designed for rearing newly transformed mussels while conducting chronic (≥28 day) toxicity trials, and can be easily enhanced with a pumping system to continuously deliver chemical solutions allowing chronic toxicity assessments of waterborne chemicals. In the test system, survival of 1 day post transformation *Lampsilis siliquoidea* after 28 days was 80%, the typical minimum survival for 2-4 month old mussels in a control treatment group during toxicity tests conducted for 10 to 28 days (ASTM 2006).

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Evolutionary radiation of present-day *Nautilus* and *Allonautilus*

James Bonacum[1], Neil H. Landman[2], Royal H. Mapes[3], Matthew M. White[3], Alicia-Jeannette White[1], and Justin Irlam[1]

[1] Department of Biology, University of Illinois at Springfield, Springfield, Illinois 62703, U.S.A.

[2] American Museum of Natural History, New York, New York 10024, U.S.A.

[3] Department of Geological Sciences, Ohio University, Athens, Ohio 45701, U.S.A.

Correspondence, J. Bonacum: jbona1@uis.edu

Abstract: The extensive fossil record of coiled nautiloids indicates that they comprised a diverse assemblage of species in ancient oceans. Today they are represented by the genera *Nautilus* Linnaeus, 1758 and *Allonautilus* Ward and Saunders, 1997, inhabiting reef systems throughout the Indo-Pacific region. Some individual populations of *Nautilus* show subtle differences in shell morphologies, and these morphological differences may be used to diagnose different species. An alternative view is that these differences are simply geographically localized, morphological variants within the broadly distributed taxon generally referred to as *Nautilus pompilius* Linnaeus, 1758. Here we present a hypothesis for the phylogeny of present-day *Nautilus* and *Allonautilus* using molecular characters from two mitochondrial gene regions, 16S rDNA and Cytochrome Oxidase *c* subunit I. Populations of *N. pompilius* in Indonesia (Ambon Strait), the Philippines, Vanuatu (New Hebrides Islands), Papua New Guinea, Carter Reef and Osprey Reef (Great Barrier Reef, Queensland, Australia) and *N. macromphalus* (Sowerby, 1849) in New Caledonia were surveyed as well as samples of *N. repertus* (Sowerby, 1849) (Rowley Shoals, Western Australia), *N. belauensis* (Saunders, 1981) (Palau), and *N. stenomphalus* (Sowerby, 1849) (Queensland, Australia). The gastropod *Crepidula striolata* (Menke, 1851) was included as an outgroup. Our results indicate that *Nautilus* is currently undergoing a period of evolutionary radiation throughout the Indo-Pacific region. The topology of the strict consensus tree suggests that the basal divergence between *Nautilus* and *Allonautilus* occurred in the waters surrounding the present-day island of New Guinea and the northern part of the Great Barrier Reef in northeast Australia. This was followed by a migration to the east by the common ancestor of *N. macromphalus* and the *N. pompilius* populations in Vanuatu, Fiji, and American Samoa. A subsequent migration to the west led to the founding of populations off the west coast of Australia, the Philippines, Palau, and Indonesia. Our results also indicate that *N. macromphalus* and *A. scrobiculatus* are phylogenetic species. However, *N. pompilius* is a paraphyletic assemblage of populations and does not represent a true phylogenetic species. Divergences within the genus *Nautilus* appear to be driven by geographic isolation, and we discuss how this may be a result of constraints on dispersal imposed by the ecology of the animals.

Key words: cephalopod phylogeny, speciation, phylogeography

During most of the Phanerozoic, the world's oceans were inhabited by a vast array of externally shelled cephalopods. Today, fewer than a dozen species survive. These animals, comprising the genera *Nautilus* Linnaeus, 1758 and *Allonautilus* Ward and Saunders, 1997 are of considerable interest to both evolutionary biologists and paleontologists because they serve as model organisms for understanding the general biology of extinct nautiloids. A survey of the fossil record indicates that nautilids, the restricted group containing *Nautilus* and *Allonautilus*, experienced a series of radiations and extinctions throughout their history (Ward 1988). Although nautiloids are well represented in the fossil record prior to the Miocene, the more recent fossil record contains few representatives (Eocene–Saunders *et al.* 1996; Miocene–Teichert and Matsumoto 1987; Lower Pleistocene–Wani *et al.* 2007). The rarity in the fossil record during the past 20 million years, of a once abundant lineage of nautilids persisting to the present day suggests that populations of nautilids must have been small during this period. This is also suggested by low levels of genetic divergence in living nautilids (Woodruff *et al.* 1987, Wray *et al.* 1995, Bonnaud *et al.* 2004).

Although the fossil record of nautilids has been explored in detail (Miller 1947, Kummel 1956, Matsumoto 1983, Tintant and Kabamba 1983), little is known about the relationships of the extant forms, and the actual number of species and genera is a matter of debate. Eleven species have been proposed, and at least seven are thought to be synonymous: *Nautilus pompilius* Linnaeus, 1758 (= *N. ambiguus* Sowerby, 1849 = *N. alumnus* Iredale, 1944); *N. scrobiculatus* Lightfoot, 1786 (= *N. umbilicatus* Lister, 1685 = *N. perforatus* Conrad, 1849 = *N. texturatus* Gould, 1857); *N. macromphalus* Sowerby, 1849; *N. stenomphalus* Sowerby, 1849; *N. repertus* Iredale, 1944; and *N. belauensis* Saunders, 1981. Saunders (1987) concluded that there are only four recognizable species: *N. pompilius*, *N. macromphalus*, *N. scrobiculatus*, and *N. belauensis*. He regarded the status of *N. repertus* and *N. stenomphalus* as questionable. Ward and Saunders (1997) proposed the new genus *Allonautilus* to

group together *N. scrobiculatus* and *N. perforatus*, based on the extreme morphological differences of these species relative to all other extant species, as well as the levels of genetic divergence reported by Wray *et al.* (1995) and Woodruff *et al.* (1987). The validity of this genus has been subject to debate (Harvey *et al.* 1999, Ward 1999). We provisionally adopt the usage of Ward and Saunders (1997). To avoid confusion, we refer to the animals in both genera as nautilus with a lowercase "n" and non-italicized. When we refer to the genus *Nautilus*, we use uppercase and italics.

Part of the taxonomic confusion regarding nautilus results from the fact that all of the proposed species, with the exception of *Nautilus belauensis*, were originally described from drift shells collected on beaches throughout the Indo-Pacific (Fig. 1). Specimens that possessed unique shell morphologies were identified as new species, but the degree of morphological variation within populations was unknown. Because nautilus shells can be transported great distances by surface currents, making it virtually impossible to ascertain their point of origin (Saunders and Spinosa 1979, House 1987), it was difficult to assess whether morphological differences in shells used for species diagnoses were fixed within populations, making them appropriate diagnostic characters (*sensu* Davis and Nixon 1992), or variation within a single species with a broad geographic distribution.

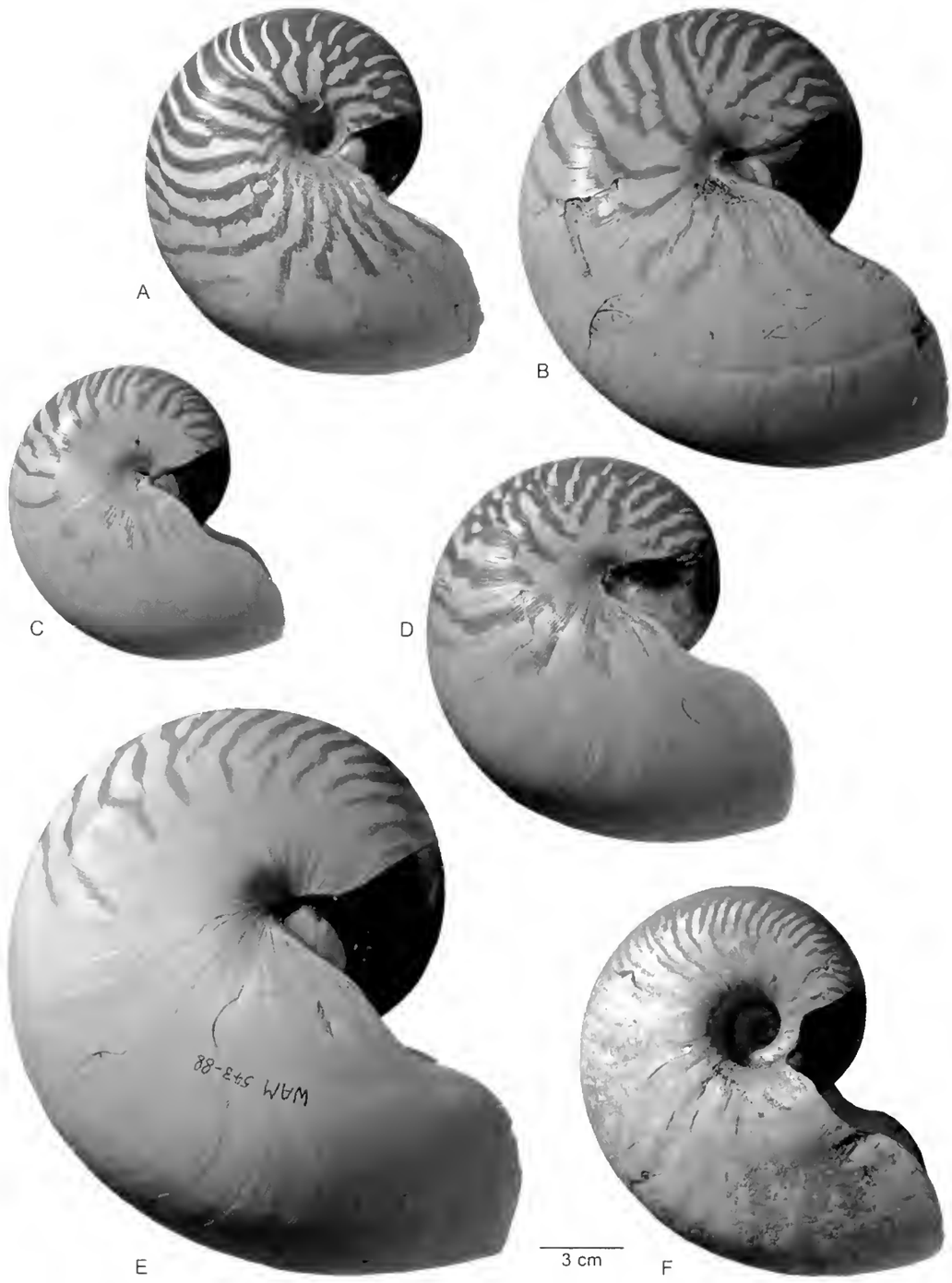

Figure 1. Specimens of the shells of various putative species of Nautilus. A, *Nautilus macromphalus* Sowerby, 1849, New Caledonia (AMNH 3078570). B, *Nautilus belauensis* Saunders, 1981a, Palau (AMNH 43027). C, *Nautilus stenomphalus* Sowerby, 1849, Carter Reef, Australia (AMNH 43260). D, *Nautilus pompilius* Linnaeus, 1758, Papua New Guinea (AMNH 43028). E, *Nautilus repertus* Iredale, 1944, northwest Australia (AMNH 58545). F, *Allonautilus scrobiculatus* Lightfoot, 1786, Papua New Guinea (AMNH 43261).

The first information regarding the distribution of the various species that had been proposed can be found in the work of Arthur Willey, who trapped specimens in and around New Guinea and New Caledonia from 1894 to 1897 (Willey 1899, 1902). Willey's results, along with those of subsequent expeditions in the 1970s and 1980s indicated that four species previously proposed based on shell morphology (*Nautilus macromphalus, N. belauensis, N. stenomphalus*, and *Allonautilus scrobiculatus*) were localized to particular reef systems (Saunders 1981a,1981b, Saunders and Ward 1987a, 1987b, Ward 1988). *Nautilus macromphalus* occurs in New Caledonia and the Loyalty Islands, *N. belauensis* on reefs near Palau, *N. stenomphalus* off the coast of Queensland near the Great Barrier Reef in Australia, and *Allonautilus scrobiculatus* in Papua New Guinea. In contrast, *N. pompilius*, the type species for the genus, appears to have a much broader distribution. The documented western limit of its range is the

Andaman Islands of Indonesia, although individuals have been collected as far west as the Seychelles and Mauritius in the Indian Ocean. The eastern extreme is American Samoa. To the south, drift specimens have been collected in New Zealand and on the coast of New South Wales in Australia. Populations of *N. pompilius* occur throughout the Philippines; these appear to represent the northernmost limit of the distribution of present-day nautilus (Saunders 1987).

Wray *et al.* (1995) performed a phylogenetic analysis of *Nautilus* and *Allonautilus* using 10 morphological characters and mitochondrial and nuclear DNA sequences. Their results indicated low levels of sequence divergence, and they recovered only two phylogenetic species (*sensu* Cracraft 1983), *A. scrobiculatus* and *N. pompilius*. The *A. scrobiculatus* samples formed a monophyletic group that was the sister group to the genus *Nautilus*. *Nautilus* in turn contained three clades that corresponded to the biogeographic distributions of the populations and species included in the study, which the authors designated the Western Pacific, Australia/Papua New Guinea, and Western Australia/Indonesia clades. Each clade contained representatives of different populations of *N. pompilius* collected in each of these areas, as well as a representative of one of the other proposed species collected in the same geographic region. For example, the Western Pacific clade consisted of *N. pompilius* specimens collected in Fiji and American Samoa along with a specimen of *N. macromphalus* collected in New Caledonia. Wray *et al.* (1995) concluded that *N. belauensis, N. macromphalus,* and *N. stenomphalus* were morphological variants of a broadly distributed *N. pompilius*. While this study suggested the existence of only two phylogenetic species, it was also consistent with the hypothesis that present-day nautilus are currently undergoing a period of diversification (Saunders 1981b, 1987, Ward 1984, Teichert and Matsumoto 1987, Woodruff *et al.* 1987).

Bonnaud *et al.* (2004) observed substantial differences between the 18S rRNA sequences of *Allonautilus scrobiculatus* and those of *Nautilus macromphalus* and *N. pompilius*, supporting the validity of *Allonautilus*. There was very little sequence divergence between *N. pompilius* and *N. macromphalus,* and the authors agreed with Wray *et al.* (1995) that the distinctive shell of *N. macromphalus* was most likely a geographic variant of *N. pompilius*.

These studies clearly suggest that *Allonautilus scrobiculatus* represents an evolutionary lineage that is separate from the genus *Nautilus*. However, the number of species in the genus *Nautilus* remains unclear, as does the taxonomic status of *N. pompilius*. In addition, previously published phylogenetic studies either did not include an outgroup (Wray *et al.* 1995) or were unable to resolve the phylogenetic relationships within the genus *Nautilus* (Ward and Saunders 1997).

We address the issue of speciation in *Nautilus* and *Allonautilus* using data for another mitochondrial gene region, Cytochrome Oxidase *c* subunit I (COI), for all of the taxa included in the Wray *et al.* (1995) study, as well as an additional 48 specimens representing *N. pompilius* collected from seven different localities in the Indo-Pacific, and *N. macromphalus* collected from two different localities in New Caledonia (Fig. 2). This gene region was selected for its utility as a source of characters for phylogenetic analyses (Remigio and Hebert 2003) due to its tendency to show low levels of intraspecific variation and high levels of interspecific variation. In addition, this particular gene region has recently attracted a great deal of attention due to its use in molecular barcoding studies (Hebert *et al.* 2003a, 2003b, Ratnasingham and Hebert 2007). We collected these data, as well as additional 16S rDNA (16S) sequence data, for all of the additional samples and then analyzed the combined and individual molecular data sets using maximum parsimony. We also conducted a Population Aggregation Analysis (PAA) (Davis and Nixon 1992) to identify suites of diagnostic molecular characters for individual populations and species and analyzed the biogeographic distributions of the various species and populations included in the study.

We address the following questions. What is the relationship of *Allonautilus* to other present-day nautilus? Are *Nautilus macromphalus, N. stenomphalus, N. belauensis,* and *N. repertus* distinct phylogenetic species or are they morphological variants of *N. pompilius*? Is *N. pompilius* a single widespread morphologically variable species, or is it a paraphyletic assemblage consisting of populations that belong to different evolutionary lineages? Can the biogeographic distribution of nautilus populations be related to their evolutionary history? And, lastly, do the phylogeny and ecology of present-day nautilus suggest a possible explanation for the patterns of extinction and radiation seen in the fossil record of the nautilids?

MATERIALS AND METHODS

The specimens used in this study were collected over a period of 20 years by several individuals. At the time of collection the specimens were either frozen or preserved in 70% ethanol. Tissue samples for the specimens collected in New Caledonia and Vanuatu have been deposited in the Invertebrate Zoology collections at the American Museum of Natural History. The associated DNA samples extracted from these specimens have been archived in the Ambrose Monell Cryo Collection at the American Museum of Natural History and at the University of Illinois Springfield. All other tissue and associated DNA samples are archived at the University of Illinois Springfield. Detailed collection information is provided in the Appendices along with the AMNH and UIS sample numbers and the GenBank Accession numbers for the corresponding sequences.

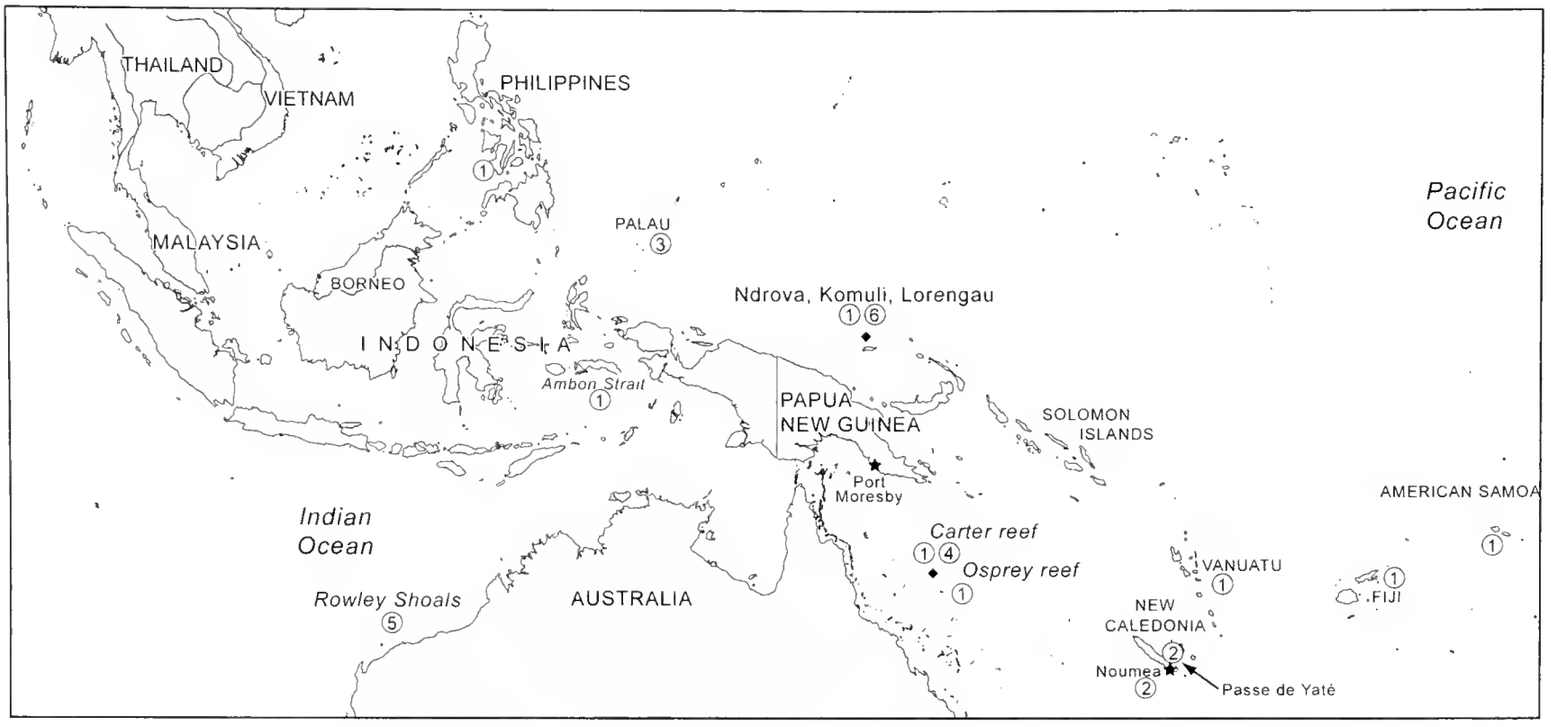

Figure 2. Distribution of nautilus populations included in this study. ①, *N. pompilius*; ②, *N. macromphalus*; ③, *N. belauensis*; ④, *N. stenomphalus*; ⑤, *N. repertus*; ⑥, *A. scrobiculatus*. Sites where individuals of two species occur sympatrically are indicated with a "♦". For collection information see Table 1.

DNA extraction, amplification and sequencing

Extraction of DNA was carried out on either mantle or jaw muscles using the DNAEasy kit (Qiagen) according to the manufacturer's instructions. Extracts were stored at -20 °C for short-term usage. A 412 base pair (bp) region of the Cytochrome Oxidase c subunit I (COI) gene, corresponding to bases 160-572 in the published genome of *Nautilus macromphalus* (Boore 2006, GenBank NC007980), was amplified by the Polymerase Chain Reaction (PCR) using the HCO and LCO primers of Folmer *et al.* (1994). The 399 bp fragment of the 16S rDNA gene, corresponding to bases 2,836 to 3,236 in the *N. macromphalus* genome, was amplified using the 16sa and 16sb primers (Palumbi 1996). Although the original amplicons were larger for both fragments, the actual data sets used for the various analyses are somewhat shorter. This is because the length of readable sequence varied among the different fragments. If we had used the complete sequences, we would have had to include a substantial amount of missing data. Consequently, we trimmed the ends of the sequences to a standard length in order to obtain the largest possible data set that included all characters for each of the samples. Standard reagents were used, and the reactions were carried out in a volume of 25 µl. Amplification conditions for both gene regions were 5 minutes at 94 °C, followed by 40 cycles of 30 seconds at 94 °C, 30 seconds at 46 °C, and 30 seconds at 72 °C, with a final extension step of 7 minutes at 72 °C. PCR products were visualized by agarose gel electrophoresis, and amplification products were purified using AMPure beads (Agencourt Corp.) according to the manufacturer's instructions. Sequencing reactions were performed in both directions with the amplification primers using BigDye v1.1 (Applied Biosystems) according to the manufacturer's instructions. Cycle sequencing products were precipitated with isopropanol, cleaned with 70% ethanol, resuspended in formamide, and read by an ABI Prism 377 DNA Sequencer. Sequence reads from the 5' and 3' directions were assembled into contigs and edited using the computer program Sequencher v4.6 (Gene Codes Corporation). The edited sequences were combined with previously published 16S sequences from Wray *et al.* (1995) and aligned using Sequencher 4.6. In several instances, this gene region was re-sequenced from new DNA extracts prepared from the same specimens to clarify ambiguities in the published sequences. The alignment was verified by visual inspection. Alignments were trivial, and only a single indel occurs within the 16S sequence. The gastropod species *Crepidula striolata* (Menke, 1851) was included as an outgroup to root the phylogeny. The GenBank accession numbers for the *Crepidula* sequences were AF545972.1 (16S) and AF353123.2 (COI).

Phylogenetic analysis

The aligned sequences were exported from Sequencher 4.6 as nexus files. The 16S and COI files were analyzed individually and also combined for the purpose of performing

a simultaneous analysis (SA) (Nixon and Carpenter 1996) using PAUP v.4.0b3 (Swofford 2003). Due to the large number of taxa included in the analysis, the heuristic search algorithm was employed with the tree bisection reconnection (TBR) branch swapping option. Maximum parsimony was chosen as the optimality criterion. One hundred random stepwise additions were performed. PAUP also was used to calculate bootstrap values (BV) for the entire data matrix by performing 1000 bootstrap replicates with 10 random additions of taxa per replicate. Partitioned Bremer support values (BS; Bremer 1992, Baker *et al.* 1998) were calculated with the computer program TreeRot v2b (Sorenson 1999) using the strict consensus tree recovered from the SA. Prior to performing the bootstrap and Bremer support analyses, all redundant taxa (individuals with identical haplotypes) were deleted from the analysis with the exception of the two representatives of the Fiji population. Both of these individuals were included in order to determine bootstrap and Bremer support values for this population.

Estimation of genetic distances

Uncorrected p distances were calculated for the SA data matrix using PAUP v.4.0b3 (Swofford 2003). All pair wise comparisons between individuals included in the ingroup were performed. The pair wise distances were exported into an Excel® (ver. 10) spreadsheet and average genetic distances for various subsets of the data were calculated using the descriptive statistics function in the Data Analysis tool pack.

We calculated the average genetic distance of all pair wise comparisons within both *Nautilus* and *Allonautilus,* as well as the average genetic distance from all pair wise comparisons between individuals in the two genera. We also calculated the average genetic distance for all pair wise comparisons of individuals within the Western Australia/Indonesia, Australia/Papua New Guinea, and Western Pacific clades (as defined by the results of the phylogenetic analysis). The average genetic distances among the Western Australia/Indonesia, Australia/Papua New Guinea, and Western Pacific clades were calculated by averaging the results of all pair wise comparisons of individuals in one clade with all individuals in another clade (*e.g.*, all pair wise comparisons between individuals in the Western Australia/Indonesia clade and individuals in the Australia/Papua New Guinea clade). The average genetic distance between *Allonautilus* and each of the three biogeographic clades was calculated by taking the average of all pair wise comparisons between the three representatives of *Allonautilus* included in the study and the individuals within each of the three clades. Lastly, the average genetic distance between one of the samples collected at Lorengau, and the individuals in each of the three biogeographic clades was calculated as well as the average genetic distance between this sample and the three samples of *Allonautilus.* This particular sample was selected because Wray *et al.* (1995: 225) noted that it possessed an "unusual mt haplotype" and formed a trichotomy with the Western Pacific and Australia/Papua New Guinea clades in the results of their analysis.

Population aggregation analysis

In addition to the 67 taxa included in the SA, 25 additional 16S sequences and 60 additional COI sequences were obtained from specimens from the Ndrova and Vanuatu populations of *Nautilus pompilius*, and from individuals of *N. macromphalus* collected at two different localities in New Caledonia, Noumea, and Passe de Yaté. These individuals were not included in the SA because each was represented by only one of the two data partitions included in the SA, and their inclusion in the phylogenetic analysis would have resulted in the introduction of a substantial amount of missing data.

These additional sequences were combined with the appropriate gene regions used in the SA to perform a Population Aggregation Analysis (PAA) (Davis and Nixon 1992). This is a non-tree based approach that identifies species on the basis of diagnostic characters. Two types of characters are considered diagnostic. A pure diagnostic character is defined as one where a particular character state is fixed in a particular population or group of populations but does not occur in any other populations. Private diagnostic characters are defined by the presence of a unique character state that occurs in some, but not all individuals, in a particular population or group of populations, but do not occur in any other populations. We included the additional sequences in this analysis because the larger sample sizes provided a more rigorous test of the diagnostic abilities of the individual characters.

The PAA was performed by a visual inspection of the individual data matrices using MacClade v4.0 (Maddison and Maddison 2000). The locations and numbers of pure and private diagnostic characters (Davis and Nixon 1992) for each gene region were determined for each population, or species that was represented by more than a single individual. In the case of populations or species that were represented by a single individual (*e.g.*, *Nautilus stenomphalus*), autapomorphies were scored as private diagnostic characters.

Biogeographic analysis

In order to reconstruct the biogeographic history of the populations in the study, all 14 collection localities were coded as an unordered multistate character. The ancestral states for this character were reconstructed for each node in the strict consensus of the maximum parsimony trees under the criterion of maximum parsimony using the computer program Mesquite (Maddison and Maddison 2004).

RESULTS

Phylogenetic analysis

The data matrix consisted of 811 molecular characters. Within the ingroup, 648 characters were constant, 43 characters were parsimony uninformative, and 120 characters were parsimony informative (PI). The 16S data partition contributed 40 of the PI characters, and the COI data partition contributed 80.

The SA produced 24 most parsimonious trees (MPTs), 564 steps in length with a consistency index (CI) of 0.822, and a retention index (RI) of 0.938. The strict consensus of these trees is shown (Fig. 3). Our results indicate that *Allonautilus scrobiculatus* is the sister taxon to all other proposed species within *Nautilus,* and that the various populations/species sampled within *Nautilus* form the same three distinct clades associated with their biogeographic distribution that were originally described by Wray *et al.* (1995). Following their nomenclature, the Western Pacific clade (Node III in Fig. 3) consists of *Nautilus macromphalus* and samples of *N. pompilius* collected in Vanuatu, Fiji, and American Samoa. The Australia/Papua New Guinea clade (Node VI in Fig. 3) includes samples of *N. stenomphalus, N. pompilius*, and a specimen identified by its collectors as a hybrid of *N. stenomphalus* and *N. pompilius* that was collected at Carter Reef (identified as Lizard Island in Wray *et al.* 1995), as well as specimens of *N. pompilius* that were collected at Osprey Reef. Both of these collection sites are located on the Great Barrier Reef in Queensland. This clade also contains specimens from several localities in Papua New Guinea (PNG). These are Ndrova Island, Komuli Island, and Lorengau, which are located within the province of Manus (PNG), and Port Moresby, which is on the southern coast of the island of New Guinea. The Western Australia/Indonesia clade (Node V in Fig. 3) includes a single specimen of *N. belauensis,* two specimens of *N. repertus,* two specimens of *N. pompilius* from the Ambon Strait in Indonesia, and three specimens of *N. pompilius* that were collected in the Philippines.

The individual analyses of the 16S and COI data partitions recovered all monophyletic groups that were observed in the SA (data not shown) with the exception of the Western Australia/Indonesia clade, which was not recovered by the individual analysis of the 16S data partition. The results of the individual analyses both indicate that *Allonautilus* is the sister taxon to *Nautilus*, but neither the 16S or COI data partitions alone were able to resolve the basal relationship among the three biogeographic clades within *Nautilus*. In the individual analysis of the 16S data partition a sample collected at Lorengau that Wray *et al.* (1995) described as having a unique haplotype, was not placed within any of the three biogeographic clades, which is consistent with the results they obtained. In contrast, the individual analysis of the COI data places this specimen (asterisk in Fig. 3) at the base of the Australia/Papua New Guinea clade.

All of the placements in the SA are consistent with the results of Wray *et al.* (1995) in that we also recovered the same three biogeographic clades that they first described, and in our results the placement of individuals and specific populations are consistent with their geographic distribution. But unlike the previous study (Wray *et al.* 1995) which recovered only two phylogenetic species (*Nautilus pompilius* and *Allonautilus scrobiculatus*), the more extensive sampling and additional data in our study indicate that the genus *Nautilus* is not monospecific as previously thought. Our results indicate that *N. macromphalus* is not a morphological variant of *N. pompilius*, but a distinct phylogenetic species. Furthermore, our results indicate that *N. pompilius* is not a single broadly distributed species but a paraphyletic assemblage of populations inhabiting many different reef systems throughout the Indo-Pacific. Several of these populations form monophyletic groups in both the SA and individual analyses, suggesting that they are phylogenetic species.

The Western Pacific clade is sister to a monophyletic group consisting of the other two biogeographic clades. Within the Western Pacific clade, *Nautilus macromphalus* forms a monophyletic group. Two populations of *N. macromphalus* were sampled, one from the southwest coast of New Caledonia, near Noumea, and the other from the southeastern side of the island near the town of Yaté. Together, the samples from both populations form a single well-supported monophyletic group (BV=100; BS=13). This is not surprising as both populations are located on the reef system surrounding the island of New Caledonia and there are no barriers to migration between the two localities. The presence of identical haplotypes in both populations suggests that *N. macromphalus* represents all of the individual nautilids inhabiting the reefs surrounding the island of New Caledonia and that together these individuals comprise a single panmictic population.

Nautilus macromphalus is the sister group to a clade that contains three populations of *N. pompilius*. Two of these populations, those from Vanuatu and Fiji, are monophyletic and possess fixed diagnostic characters and exclusive haplotypes, although the sampling of the Fiji population is not as extensive as that of the population in Vanuatu. The Vanuatu clade is well supported (BV=97: BS=4), as is the Fiji clade (BV=100; BS=11). The population in American Samoa is represented by a single individual, which is placed outside of the other populations in the Western Pacific clade.

In the Western Australia/Indonesia clade, the three *Nautilus pompilius* samples from the Philippines form a monophyletic group that is sister to a clade that contains *N. pompilius* from the Ambon Strait in Indonesia, *N. repertus* from the west coast of Australia, and *N. belauensis* from Palau.

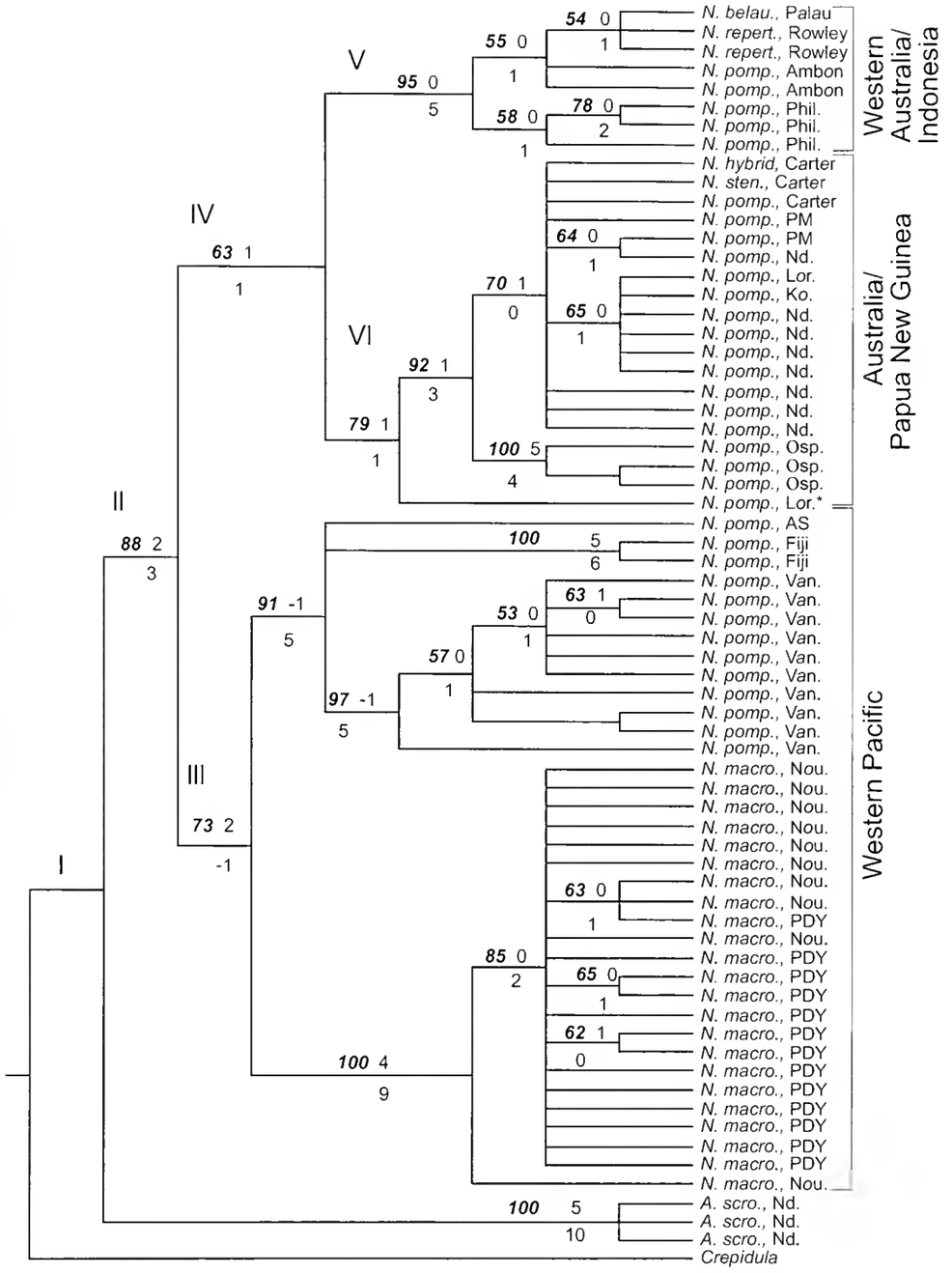

Figure 3. Strict Consensus of 24 most parsimonious trees recovered by the Simultaneous Analysis of the 16S and COI data. (Tree length = 564 steps, Consistency Index = 0.822, Retention Index = 0.938). Numbers in bold text above the branches at nodes represent bootstrap values. Numbers in plain text above the branches at nodes represent the Bremer Support contributed by the 16S data partition. Numbers in plain text below the branches at nodes represent the Bremer support contributed by the COI data partition. The sum of the Bremer support contributed by the individual data partitions at each node is the total Bremer support at that node. Roman numerals refer to specific nodes discussed in the text. (Abbreviations: AS, American Samoa; Carter, Carter Reef, Queensland, Australia; Ko, Komuli Island, PNG; Lor, Lorengau, Manus, PNG; Nd, Little Ndrova Island, PNG; Nou, Noumea, New Caledonia; Osp, Osprey Reef, Queensland, Australia; PDY, Passe de Yaté, New Caledonia; Phil, Philippines; PM, Port Moresby, PNG; Rowley, Rowley Shoals, W. Australia; Van, Vanuatu).

Support for the Philippine clade is weak (BV=58; BS=1) and is provided entirely by the COI data. Note that the two *N. repertus* samples form a weakly supported (BV=54; BS=1) polytomy with the single *N. belauensis* sample.

The Australia/Papua New Guinea clade contains three groups. There is a single well supported monophyletic group (BV=100; BS=9) consisting of 3 samples collected at Osprey Reef in Queensland. This population is the sister group to a larger clade consisting of samples collected at various sites in and around the island of New Guinea and Carter Reef. The Carter Reef specimens purportedly represent two species of *Nautilus*, which have been reported to occur sympatrically at this location (*N. pompilius* and *N. stenomphalus*), and an individual that was identified as a hybrid of the two. At the base of the entire Australia/Papua New Guinea clade is the individual collected at Lorengau, on Manus Island, Papua New Guinea, that formed a trichotomy with the Western Australia/Indonesia and Australia/Papua New Guinea clades in the results obtained by Wray *et al.* (1995). Support for this placement and the entire Australia/Papua New Guinea clade is relatively weak (BV=79; BS=2). Support for the node that includes the rest of the individuals assigned to the Australia/Papua New Guinea clade is high (BV=92; BS=4).

Genetic distance estimates

The average of the genetic distances between the various populations are reported (Table 1). The average genetic distance between individuals within the Western Pacific clade was 3%, which is three times higher than the average distances between the individuals in the other two biogeographic clades (1%). Also, the average genetic distance between the Western Pacific clade and the other two biogeographic clades (6% in both cases) was higher than the average genetic distance between the Western Australia/Indonesia and Australia/Papua New Guinea clades (4%). The average genetic distance between

Table 1. Genetic distance data. The average genetic distances between populations and/or individuals collected at each of the localities included in the study expressed as uncorrected p distances.

	Nautilus belauensis	*Nautilus repertus*	*Nautilus pompilius* Ambon Strait, Indonesia	*Nautilus pompilius* Philippines	*Nautilus pompilius* Komuli Island, Papua New Guinea	*Nautilus pompilius* Lorengau, Papua New Guinea	*Nautilus pompilius* Ndrova Island, Papua New Guinea
Nautilus belauensis	—						
Nautilus repertus	0.003	—					
Nautilus pompilius Ambon Strait	0.003	0.004	—				
Nautilus pompilius Philippines	0.010	0.012	0.008	—			
Nautilus pompilius Komuli Island, Papua New Guinea	0.036	0.040	0.036	0.038	—		
Nautilus pompilius Lorengau, Papua New Guinea	0.041	0.045	0.041	0.044	0.019	—	
Nautilus pompilius Ndrova Island, Papua New Guinea	0.035	0.039	0.035	0.036	0.003	0.018	—
Nautilus pompilius Osprey Reef, Queensland, Australia	0.042	0.045	0.042	0.042	0.020	0.032	0.018
Nautilus pompilius Port Moresby, Papua New Guinea	0.035	0.038	0.035	0.036	0.004	0.019	0.002
Nautilus stenomphalus	0.037	0.040	0.038	0.039	0.006	0.040	0.005
Nautilus macromphalus	0.059	0.063	0.057	0.061	0.058	0.063	0.057
Nautilus pompilius Vanuatu	0.056	0.060	0.057	0.059	0.059	0.063	0.058
Nautilus pompilius Fiji	0.063	0.062	0.061	0.066	0.063	0.068	0.062
Nautilus pompilius American Samoa	0.058	0.059	0.057	0.059	0.063	0.066	0.062
Allonautilus scrobiculatus	0.066	0.069	0.067	0.069	0.068	0.072	0.066

Table 1. (Continued)

Nautilus pompilius Osprey Reef, Queensland, Australia	*Nautilus pompilius* Port Moresby, Papua New Guinea	*Nautilus stenomphalus*	*Nautilus macromphalus*	*Nautilus pompilius* Vanuatu	*Nautilus pompilius* Fiji	*Nautilus pompilius* American Samoa	*Allonautilus scrobiculatus*
0.012	—						
0.021	0.005	—					
0.061	0.057	0.060	—				
0.059	0.058	0.061	0.052	—			
0.063	0.062	0.064	0.056	0.038	—		
0.063	0.062	0.064	0.056	0.035	0.037	—	
0.070	0.066	0.069	0.077	0.074	0.081	0.080	—

Allonautilus and the Western Pacific clade was 8% which was slightly higher than the 7% average genetic distance between the three representatives of *Allonautilus* and all 63 members of the genus *Nautilus* included in the study. The average genetic distance between *Allonautilus* and the other two biogeographic clades was 7% for both.

Wray *et al.* (1995: 225) reported that the sequence of one of the samples of *Nautilus pompilius* included in their study, that had been collected at Lorengau, in Papua New Guinea, had "numerous autapomorphic substitutions as well as synapomorphies with the other clades." We estimated the average genetic distance between this individual and each of the three biogeographic clades. Our results indicate that the average genetic distance between this individual and the other clades was 7% when it was compared with the Western Pacific clade, a distance as great as the average genetic distance between *Allonautilus* and *Nautilus*. The average genetic distance between this individual and the Western Australia/Indonesia clade was 5%. Comparisons to the other individuals within the Australia/Papua New Guinea clade indicate the average genetic distance between this individual and the other members of the clade was 4%. This is almost as large as the average genetic distance between this individual and the Western Australia/Indonesia clade. It is also four times the average distance obtained from the other pairwise comparisons among individuals placed within the Australia/Papua New Guinea clade.

Population aggregation analysis

The results of the PAA are in agreement with the results of the SA and the individual analyses in that each of the monophyletic groups recovered by the phylogenetic analysis that corresponded to individual populations also possesses a suite of pure and private diagnostic characters (see Table 2). A total of 41 pure and 25 private diagnostic characters were recovered from both data partitions. The number of both types of diagnostic sites was slightly higher for the COI data partition, which accounted for 51% (21/41) of the pure and 54% (13/25) of the private diagnostic sites.

It was not possible to determine whether the *Nautilus pompilius* populations in American Samoa and Komuli Island, or whether *N. stenomphalus* and *N. belauensis* represent monophyletic groups because each is represented by a single individual. Autapomorphies in the sequences of these individuals therefore were counted as private diagnostic sites. The Komuli Island specimen and the single individual of *N. stenomphalus* each possess a single autapomorphy. The single individual representing the American Samoa population has 6 autapomorphies (3 in the 16S sequence and 3 in the COI sequence). All these autapomorphies may be pure or private diagnostic characters for these species or populations, but this cannot be determined without additional sampling of the populations. The *N. belauensis* sequences did not contain any autapomorphies, although they share a single pure diagnostic site with the two *N. repertus* samples, suggesting that these two putative species may be synonymous.

At the base of the Australia/Papua New Guinea clade is a specimen of *Nautilus pompilius* that was collected at Lorengau. This is the same specimen that was described as having a unique haplotype by Wray *et el.* (1995). The sequences contain 10 autapomorphies, 1 in the 16S sequence and 9 in the COI sequence.

Biogeographic analysis

The reconstruction of the ancestral character state at the nodes representing the divergence between *Allonautilus* and *Nautilus* (Node I in Fig. 3) indicates that this occurred in the waters off Papua New Guinea. This is also the case for the nodes representing the divergence events that led to the founding of each of the three clades within *Nautilus* (Nodes II and IV in Fig. 3), suggesting that the current radiation of present-day nautilus has its origin in this region. The reconstruction of the ancestral

Table 2. Results of the Population Aggregation Analysis.

	Number of diagnostic characters				
Clade	N COI	COI partition	N 16S	16S partition	Total
Allonautilus scrobiculatus	3	11	3	9	14
Nautilus macromphalus	83	9 (7 PRIVATE, 2 PURE)	38	5 (2 PRIVATE, 3 PURE)	14
Nautilus pompilius Vanuatu	13	5 (2 PRIVATE, 3 PURE)	14	8 (7 PRIVATE , 1 PURE)	13
Nautilus pompilius Fiji	2	4	2	3	7
Nautilus pompilius Osprey Reef	5	3 (1 PRIVATE, 2 PURE)	4	3	6
Nautilus pompilius Philippines	3	3 (PRIVATE)	3	3 (PRIVATE)	6

N COI and N 16S indicate the number of sequences for each species or population that was included in the Population Aggregation Analysis. Numbers in the COI partition and 16S partition columns indicate the number of diagnostic characters present in each data partition for each species or population. All characters are pure diagnostic characters (*sensu* Davis and Nixon 1992) unless otherwise indicated.

character states also indicates that the divergence represented by the basal node in the Australia/Papua New Guinea clade (Node VI) also occurred in Papua New Guinea.

The topology of the strict consensus tree indicates that the first divergence in *Nautilus* (Node II in Fig. 3) occurred when the common ancestor of the Western Pacific clade diverged from the common ancestor of the Australia/Papua New Guinea and Western Australia/Indonesia clades, presumably as a result of a migration to the east. The basal divergence within the Western Pacific clade (Node III in Fig. 3) occurred when *N. macromphalus* diverged from the common ancestor of the Fiji, Vanuatu, and American Samoa populations. If this occurred as a result of a migration from New Caledonia to Vanuatu followed by subsequent migrations to Fiji, and finally American Samoa, it would be consistent with a pattern of progressive dispersal to the east. The reconstruction of the ancestral character state at this node is equivocal though and we cannot at this time determine the order in which these populations were founded.

The migration to the east was followed by a second migration from Papua New Guinea, this time to the west by the ancestor of the Western Australia/Indonesia clade (represented by Node IV in Fig. 3). The ancestral character state reconstruction at the node representing the basal divergence within this clade (Node V in Fig. 3) is also equivocal, and we are unable to determine the order in which the four locations represented in this clade (Ambon Strait, Rowley Shoals, Palau, and the Philippines) were colonized.

DISCUSSION

Our results suggest that the present-day nautilids are undergoing a period of evolutionary radiation throughout the Indo-Pacific region. The ancestral character state reconstruction provided by the biogeographic analysis indicates that the divergence between *Allonautilus* and *Nautilus* (Node I in Fig. 3) occurred in the waters off Papua New Guinea. This is consistent with the observation that *A. scrobiculatus* and *N. pompilius* both occur at Ndrova Island (Saunders *et al.* 1987a). Also, the ancestral character state reconstructions at the nodes representing the migrations that led to the founding of the Western Pacific clade (Node II in Fig. 3), and its divergence from the common ancestor of the Western Australia/Indonesia and Australia/Papua New Guinea clades (Node IV in Fig. 3) indicate that these divergences also occurred in this region. Papua New Guinea lies at the geographic center of the distribution of all present day nautilus. For these reasons, we hypothesize that the waters off Papua New Guinea provided a refuge for the survivors of the events that led to the sudden decline of nautilids in the fossil record following the end of the Miocene.

The fact that samples collected in the western Pacific and western Australia/Indonesia regions form monophyletic groups in the topology of the strict consensus tree suggests that the evolutionary radiations in these regions may have occurred following a single founder event. While well adapted to life on sloping reef faces, present-day nautilus are rarely found in the open ocean. Saunders *et al.* (1987b) observed damage to the shell, mantle, and hood of trapped specimens that resulted from predation by octopus and fish. These authors reported direct observations of attacks on nautilus by triggerfish and groupers. The specimens that were attacked in these instances made no attempts to defend themselves beyond retracting into their shells, and nautilus in the water column appear to be easy prey for individuals of a variety of species. Attempts to avoid predation by seeking shelter along the ocean bottom are limited by cooler temperatures at depth, as well as the shell implosion depth of 600 m (Saunders and Ward 1987b). All present-day nautilus are also extremely slow swimmers, usually covering less than 2 km per day (Ward *et al.* 1984, Saunders and Ward 1987b). In order to travel significant distances across deep water basins, nautilus most likely require some form of passive transport such as an ocean current.

These limitations suggest that successful long distance migrations by nautilus occur infrequently and that, once new populations are established, gene flow between the new population and its source rarely occurs. This would lead to reproductive isolation of the new population. Our results indicate that individual populations that are separated from others by large expanses of deep water, for example in the Western Pacific clade or the Philippines, are monophyletic. In contrast, populations that are separated by shorter distances and shallower water, such as the two populations of *Nautilus macromphalus,* still show evidence of gene flow between populations.

The lack of monophyletic populations in the Australia/Papua New Guinea clade, with the exception of the one at Osprey Reef, provides another example of gene flow occurring among populations that are separated by small distances and shallow water. Swan and Saunders (1987), in a study of morphological variation among six populations of nautilus in and around Papua New Guinea, detected variation suggesting adaptation to local conditions, but the authors were unable to find any fixed variants in any of the populations they sampled. They suggested that gene flow was occurring among the populations in this region. The placement of the Port Moresby, Komuli, and Lorengau samples in the same clade in our analysis is in agreement with this, as is the lack of diagnostic molecular characters for any of the individual populations. Komuli and Ndrova are islands located just to the east of the larger island of Manus where the port city of Lorengau is located. These three locations all lie within 50 km of one another, and the waters separating Komuli and Ndrova

from Manus are less than 200 m deep, providing ample opportunity for gene flow to occur. Gene flow between the populations in Manus Island and the rest of Papua New Guinea would require migrations over deeper water, but the distance from Manus to the main island of New Guinea is approx. 350 km, considerably less than the distances that separate populations in the Western Pacific clade; the latter range from 600 to 700 km. However, direct contact among these populations may not be required. Reports of live nautilus from the reefs surrounding the islands of New Britain and New Ireland (House 1987) indicate that resident populations in these areas could provide an avenue for gene flow between the populations in Manus Island and those off the main island of New Guinea.

The inclusion of the Carter Reef samples (Great Barrier Reef) within the Australia/Papua New Guinea clade may seem surprising because a distance of 550 km separates these two locations. But the island of New Guinea is connected to the Australian mainland by the continental shelf and the depth of the water separating these locations is 200 m or less, suggesting that gene flow could occur between these locations. This implies that gene flow can occur between individual populations of nautilus from Lorengau on Manus Island in the north to the island of New Guinea, and even further south to the Great Barrier Reef across a distance of 1200 km. Woodruff *et al.* (1987) previously suggested this possibility based on an analysis of allozyme data.

The samples collected at Osprey Reef form the only monophyletic group within the Australia/Papua New Guinea clade. Osprey Reef is separated from the continental shelf by approx. 160 km across the Queensland trough, where water depths exceed 1000 m. This is less than the presumed distance that migrants would have to travel between Manus Island and New Ireland, the two closest reef systems that are separated by deep water, where specimens of nautilus are known to occur. At Osprey Reef, however, there appears to have been a successful barrier to dispersal. Osprey Reef is a comparatively small island, only 25 km in length, compared to the 325 km length of New Ireland. Consequently, Osprey Reef is a much smaller target for an animal drifting in an ocean current to reach by chance, and the nautilus population at Osprey Reef may have been established by a single colonization event.

A single individual identified as *Nautilus stenomphalus* is placed within the Australia/Papua New Guinea clade. It was collected at Carter Reef, along with a specimen identified as *N. pompilus* and a putative hybrid of these two species. The relationship of these three specimens to one another as well as to the other individuals within this clade remains unresolved.

At the base of the Australia/Papua New Guinea clade is the individual collected at Lorengau that was identified as having a unique haplotype by Wray *et al.* (1995). It is sister to all other individuals within this clade, and the sequence divergence between this individual and all other members of the Australia/Papua New Guinea clade is 4%. In contrast, the average uncorrected p distance of all pairwise comparisons between the other individuals within this clade is 1%. Wray *et al.* (1995) speculated that it might be a migrant from a different reef system. If this is so, its source population represents a lineage that originated as a result of the earliest divergence within the Australia/Papua New Guinea clade surveyed to date, and its migration to Lorengau suggests that gene flow can occur between these populations. Additional sampling of nautilus in this area is called for before this can be determined, but at the very least it suggests that there is much more to be learned about the biogeographic history of nautilus populations.

In the Western Australia/Indonesia clade, the three *Nautilus pompilius* specimens from the Philippines form a monophyletic group that is sister to a clade that contains *N. pompilius* from the Ambon Strait in Indonesia, *N. repertus* from the west coast of Australia, and *N. belauensis* from Palau. The two *N. repertus* samples form a weakly supported (BV=54; BS=1) polytomy with the single *N. belauensis* individual. Both of these putative species are characterized as having the largest shells within the genus, and both the 16S and COI sequences for these three individuals are extremely similar. Only two sites within the COI data distinguish one of the *N. repertus* specimens from the other two species in this clade, suggesting the possibility that *N. belauensis* may be synonymous with *N. repertus*. This is somewhat surprising in light of the fact that Rowley Shoals, the collection locality for the two *N. repertus* specimens, is over 3000 km from Palau, where *N. belauensis* occurs. These sequences are also very similar to those of the Ambon Strait samples and the Philippine samples, suggesting that diversification within the Western Australia/Indonesia clade is either occurring very slowly or has begun more recently relative to the Western Pacific clade.

The fact that we did not include morphological characters in our study should not be misinterpreted to mean that we do not recognize the importance of these data. The authors of previous studies who utilized morphological data were unable to resolve phylogenetic relationships of nautilus beyond differentiating between *Allonautilus* and *Nautilus* (Wray *et al.* 1995, Ward and Saunders 1997). Of the ten morphological characters analyzed by Wray *et al.* (1995), all were phylogenetically uninformative although six of them were diagnostic for *A. scrobiculatus*. Ward and Saunders (1997) expanded on this study and analyzed a series of 20 morphological characters for *N. pompilius*, *N. macromphalus*, *N. belauensis*, *A. scrobiculatus*, and *A. perforatus*, as well as eight fossil nautilids. None of these characters resolved the phylogenetic relationships of the extant species although a subset of these characters served to separate *A. scrobiculatus*

and *A. perforatus* from *N. macromphalus*, *N. belauensis*, and *N. pompilius*, and two characters were diagnostic for *N. stenomphalus*. We recognize that a detailed comparative study of the morphology of different populations is called for to assess patterns of morphological variation.

The lack of phylogenetic information contained in the morphological characters analyzed to date may be due to a genuine lack of variation indicative of a series of recent divergences, or the fact that these characters have yet to be explored in sufficient detail. At the present time, the available morphological characters are unable to resolve the phylogeny of *Nautilus* and *Allonautilus*. The identification of phylogenetically informative morphological characters in present-day nautilus remains one of the greatest challenges in the study of these animals.

Our results indicate that *Allonautilus* is a sister taxon to *Nautilus*. This is in agreement with Wray *et al.* (1995) and Ward and Saunders (1997), and we concur that *Allonautilus* should be recognized as a separate genus within the Nautilidae. Also, *N. macromphalus* is an independent evolutionary lineage within *Nautilus* and is, therefore, a valid phylogenetic species. In contrast, *N. pompilius* is a paraphyletic assemblage of nautilus populations found throughout the Indo-Pacific. The phylogenetic hypothesis represented by the strict consensus tree we recovered from our data indicates that the nautilus populations in Vanuatu, Fiji, the Philippines, and Osprey Reef are all independent, reproductively isolated evolutionary lineages and, therefore, represent distinct phylogenetic species. Although the population at American Samoa is represented by a single individual, the large number of autapomorphies it possesses, as well as its geographic isolation, suggests that this population also may represent a phylogenetic species.

The fact that different populations of *Nautilus pompilius* may represent distinct phylogenetic species has been suggested by previous studies. For example, a significant degree of genetic differentiation between populations of *N. pompilius* in the Philippines and in Fiji was reported by Masuda and Shinomiya (1983). A comparison of biomineralization characteristics of specimens from these two populations indicated differences in magnitude as great as those attributed to interspecific differences between individual representatives of *N. pompilius*, *N. macromphalus*, and *Allonautilus scrobiculatus* (Crick and Mann 1987). The allozyme study of Woodruff *et al.* (1987) showed that the genetic distance separating individuals of *N. pompilius* collected in Fiji from supposedly conspecific individuals from localities in Papua New Guinea is sufficient to assign them to separate species. Wray *et al.* (1995), despite the fact that they were able to distinguish only two phylogenetic species due to the limited sampling in their study, also considered this possibility. We conclude that *N. pompilius*, over the years, has become a "catch all taxon" to which all specimens lacking a distinctive morphological difference (*e.g.*, the open umbilicus of *N. macromphalus*) have been assigned. This underscores the need for a more detailed study of nautilus morphology, which may reveal subtle differences between individual populations identified as *N. pompilius*.

The taxonomic status of *Nautilus belauensis*, *N. stenomphalus*, and *N. repertus* remains unclear at this time. Our results suggest that *N. belauensis* and *N. repertus* may be synonymous, but more extensive sampling is called for before this determination can be made, especially in light of the large geographic distance that separates the localities where these individuals were collected. Although the distance between Palau and the west coast of Australia is vast, there are many small islands that lie between them, and the possibility that *N. repertus* and *N. belauensis* may maintain reproductive contact through intermediate populations within these islands awaits further exploration.

The topology of the strict consensus tree indicates that a population of present-day nautilus in the Papua New Guinea region may have been the source from which all present-day nautilus were derived. We hypothesize that speciation is occurring allopatrically as a result of a series of founder events. The monophyletic groups our study recovered that correspond to individual collection localities in Fiji, Vanuatu, New Caledonia, Osprey Reef, and the Philippines support this. Our results also indicate that the extant species of nautilus seem to be recovering from their decline at the beginning of the Miocene. This may be a direct result of the fact that their adaptation to life on reef faces limits their ability to disperse. This limitation on dispersal suggests that successful migrations rarely occur. But on the occasions when they do, these same limitations also lead to reproductive isolation, and the formation of new species. This presents one possible explanation for the alternating periods of diversification and decline seen in the fossil record of nautilids.

Many aspects of the evolution and biogeography of present-day nautilus await further study. Greater sampling of individual populations undoubtedly will help to develop a more detailed picture of the dispersal and evolution of present-day nautilus. Of particular interest is the phylogenetic placement of *Allonautilus perforatus*, which at the present time is known only from drift shells. This species, which Ward and Saunders (1997) assigned to *Allonautilus* on the basis of its shell morphology, is thought to inhabit the reefs in the vicinity of Bali, Indonesia. If it is indeed a member of the same lineage as *A. scrobiculatus*, the biogeographic and evolutionary history of present-day nautilus may have been much more complex than previously imagined. Finally, the incorporation of morphological data for present-day nautilus, based on a careful, exhaustive analysis, may well provide insights into the significance of the morphological features of

fossil nautilids, providing fresh perspectives on the systematics and phylogenetic history of this group.

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Appendix 1. Samples archived at the American Museum of Natural History.

Species	AMNH specimen #	GenBank accession #		Collection location	Collection date
		COI	16S		
Nautilus macromphalus	185260	GQ280217	GQ280151	Noumea, New Caledonia	October 2003
	185263	GQ280218	GQ280152		
	185264	GQ280219	GQ280153		
	185266	GQ280220	GQ280154		
	185267	GQ280221	GQ280155		
	185269	GQ280222	GQ280156		
	185270	GQ280223	GQ280157		
	185272	GQ280224	GQ280158		
	185273	GQ280225	GQ280159		
	185275	GQ280226	GQ280160		
	185360	GQ280227	GQ280161	Passe de Yaté, New Caledonia	January 2004
	185361	GQ280228	GQ280162		
	185362	GQ280229	GQ280163		
	185365	GQ280230	GQ280164		
	185368	GQ280231	GQ280165		
	185369	GQ280232	GQ280166		
	185373	GQ280233	GQ280167		
	185374	GQ280234	GQ280168		
	185381	GQ280235	GQ280169		
	185383	GQ280236	GQ280170		
	185384	GQ280237	GQ280171		
	185393	GQ280238	GQ280172		
	185407	GQ280239	GQ280173		
Nautilus pompilius	129515	GQ280240	GQ280174	Vanuatu	July 2004
	129517	GQ280241	GQ280175		
	129518	GQ280242	GQ280176		
	129519	GQ280243	GQ280177		
	129520	GQ280244	GQ280178		
	129521	GQ280245	GQ280179		
	129525	GQ280246	GQ280180		
	129526	GQ280247	GQ280181		
	129528	GQ280248	GQ280182		
	129530	GQ280249	GQ280183		

Appendix 2. Samples archived at the University of Illinois Springfield (UIS).

Species	UIS Sample identification #	GenBank accession # COI	GenBank accession # 16S	Collection location	Collection date
Allonautilus scrobiculatus	427	GQ280250	GQ280184	Little Ndrova Island, Papua New Guinea	June 1984
	419	GQ280251	GQ280185		November 1984
	415	GQ280252	GQ280186		June 1985
Nautilus belauensis	425	GQ280187	U11625*	Palau	N/A
Nautilus repertus	423	GQ280188	GQ280124	Rowley Shoals, Western Australia	N/A
	424	GQ280189	GQ280125		
Nautilus stenomphalus	410	GQ280196	GQ280130	Carter Reef, Queensland, Australia	December 1985
Nautilus stenomphalus/pompilius hybrid	411	GQ280195	GQ280129	Carter Reef, Queensland, Australia	
Nautilus pompilius	409	GQ280197	GQ280131	Carter Reef, Queensland, Australia	
	207	GQ280198	GQ280132	Osprey Reef, Queensland, Australia	March 2003
	208	GQ280199	GQ280133		
	209	GQ280200	GQ280134		
	400	GQ280190	GQ280125	Ambon Strait, Indonesia	August 1984
	401	GQ280191	GQ280126		
	426	GQ280192	U11626*	Pangalao Island, Philippines	N/A
	701	GQ280193	GQ280127	Balayan Bay, Philippines	N/A
	702	GQ280194	GQ280128		
	420	GQ280201	GQ280135	Port Moresby, Papua New Guinea	October 1984
	421	GQ280202	GQ280136		
	22	GQ280206	GQ280140	Little Ndrova Island, Papua New Guinea	October 1984
	26	GQ280207	GQ280141		
	28	GQ280208	GQ280142		
	24	GQ280209	GQ280143		
	21	GQ280210	GQ280144		
	11	GQ280211	GQ280145		
	12	GQ280212	GQ280146		
	24	GQ280213	GQ280147		
	406	GQ280205	GQ280139	Komuli Island, Papua New Guinea	July 1984
	407	GQ280203	GQ280137	Lorengau, Manus Island, Papua New Guinea	July 1984
	408	GQ280204	GQ280138		
	402	GQ280214	GQ280148	Pago Pago, American Samoa	July 1984
	403	GQ280215	GQ280149	Suva, Fiji	August 1986
	404	GQ280216	GQ280150		

* Accession numbers for sequences used in this study and in Wray *et al.* (1995)

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Fecundity and survival advantages of an exotic gastropod compared to a native species

Verónica Núñez

División Zoología Invertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900, La Plata, Argentina

Correspondence, V. Núñez: nmariaveronik@yahoo.com.ar

Abstract: In Argentina the exotic snail *Physa acuta* Draparnaud, 1805 is predominant in environments previously inhabited by the native species *Stenophysa marmorata* Guilding, 1828, raising the question of whether this could have occurred because of differences in survival or reproductive strategies. To analyze the life cycle of these two species, I used the horizontal–life-table method and considered the number and proportion of viable of eggs per oviposition. Although both species suffered a high degree of mortality during the first weeks after oviposition, both the rate and the force of mortality was much greater during the reproductive period, so that the survival curve was not as markedly concave as with other gastropods. *Physa acuta* survived longer than *S. marmorata*, began its reproductive period earlier, and had a longer and more continuous reproductive stage. The number of ovipositions per snail was not different between the two species; but since the mean number of eggs per oviposition was higher in *P. acuta*, fecundity was likewise higher. The increase in fecundity was accompanied by an enhancement of the mortality rate in *S. marmorata*. The percentage of viable eggs was higher in *P. acuta* than in *S. marmorata*, but fecundity increased with age in both species. Life expectancy, reproductive value, and net reproductive rate were higher in *P. acuta*. The success of the exotic species *P. acuta* in the native habitat of *S. marmorata* could be explained in part by the former's earlier sexual maturation, higher reproductive potential, and greater longevity. Further field and laboratory studies are needed to demonstrate the existence of interspecific competition between these two gastropods.

Key words: life cycle, Physidae, reproduction, survivorship, introduced species

The invasion of aquatic environments by freshwater molluscs may have a negative impact on local biodiversity (Mack *et al.* 2000, Rahel 2002) and public health because many species, especially gastropods, may be intermediate hosts for waterborne parasites (Malek 1980). The pulmonate snail *Physa acuta* Draparnaud, 1805 is an invasive species, able to disperse rapidly, colonize new areas, particularly within disturbed environments, and attain high densities (Winterbourn 1980, Brackenbury and Appleton 1993). This species has become established in many parts of the world and has impacted native snails. In South Africa, for example, *P. acuta* proved better able to exploit newly disturbed environments than the native *Bulinus tropicus* (Krauss, 1848) (Brackenbury and Appleton 1993). In New Zealand, *P. acuta* appears to have replaced the native "*Physastra variabilis*" (syn. *Glyptophysa variabilis*) (Gray, 1843) and may have contributed to a decline in this species by obstructing its feeding or causing physical injury (Winterbourn 1980). The ascendancy of *P. acuta* in Australian rivers typically follows a general decline in the native species present (Zukowski and Walker 2009).

In Argentina, *Physa acuta* has dispersed rapidly, as the species has been recorded only since the 1970s (Paraense 2005). Before that time, this species had been neither cited in the literature nor collected in the field; nevertheless, *P. acuta* has recently become not only widely distributed but also highly abundant in colonized areas (Núñez 2009). Moreover, this species has increased in environments previously inhabited by the native snail *Stenophysa marmorata* Guilding, 1828, particularly within the Río de la Plata basin. The latter species inhabited mainly lentic, shallow, vegetated areas. *Physa acuta* likewise prefers lentic limno-biotopes, though capable of colonizing a diversity of environments, including anthropogenically altered or polluted habitats. In addition, the high densities in natural populations of *P. acuta* throughout the year contrast sharply with the temporally patchy populations of *S. marmorata* (Núñez 2009).

The omnipresence of *Physa acuta* raises the question of whether this can be the result, in part, from differences in reproductive strategies or in survival compared to *Stenophysa marmorata*. Estimation of demographic rates not only is of interest to life-history theory and population ecology but also is critical for the successful conservation of native species as well as the management of exotics (Frick *et al.* 2010). The purpose of this study was therefore to examine differences in fecundity and survival between these two gastropod species under controlled laboratory conditions.

MATERIALS AND METHODS

Adult *Physa acuta* were collected from an artificial pond (at Saavedra Park) in the city of La Plata (34°55′S, 57°56′W) and *Stenophysa marmorata* from Atalaya beach, in the alluvial-plain area of the Río de la Plata (35°02′S, 57°32′W).

In the laboratory, ovipositions during the first month after collection were used to construct a life table. Individuals born on the same day were separated into 40-ml aquaria (4 individuals maximum per aquarium). The snails were kept in running water without chlorine, at 24 °C under a controlled photoperiod (12 h light, 12 h dark), and fed *ad libitum* with lettuce leaves.

To analyze the life cycle of the two species, I used the horizontal–life-table method (Rabinovich 1972, Begon *et al.* 1988, Rumi 1993), considering zero to be the day when the snails were born. The number of survivors and eggs were grouped into age classes at 7-day intervals. The following values were calculated for each age: N_x = the number of snails expressed as per thousand of the initial value N_0, l_x = survival, d_x = mortality, q_x = mortality rate, k_x = force of mortality, m_x = fecundity (all specimens were considered female because they are hermaphroditic), e_x = life expectancy, V_x = reproductive value, and R_0 = net reproductive rate.

Ovipositions were monitored daily and the number of eggs per oviposition, the total development time (from laying until hatching), the proportion of hatched eggs, the number of empty eggs, the number of embryos with malformations, and the degree of development reached by the embryos in the eggs that did not hatch were recorded. Embryos in the non-hatched eggs were classified as follows: complete with normal development, partially developed, and undeveloped from the time of laying.

In order to compare the number of eggs per oviposition, and the number of eggs and ovipositions per individual between the species, I used the one-tailed Mann-Whitney test (Zar 1996). This analysis is a nonparametric alternative to the Student's *t*-test, and the samples may be of different sizes. Accordingly, a contingency table tested whether the total number of eggs laid for N_0 (*e.g.*, the replacement rate, R_0) was the same in both species. The proportion of the hatched eggs in each of the two species was also compared through the use of the contingency tables.

The values of e_x were compared by means of the Chi-square test. Correlations between the age of the individuals and the number and size of ovipositions, the average total number of eggs, or the number of hatched eggs per individual were assessed by non-parametric Spearman's coefficients of rank correlation. Correlations between the values of m_x and those of q_x and k_x were also investigated. The program xlstat-Pro 7.5 was used for the statistical analyses.

RESULTS

Stenophysa marmorata (N_0 = 202) had a survival curve that was slightly concave (type III, Begon *et al.* 1988), and the highest mortality (d_x) occurred at week 15, when the population had decreased by more than 50% (Table 1). This species survived for only 60 weeks. Although the highest d_x was registered during the first 20 weeks, the mortality rate (q_x) increased after week 40 (Table 1). The force of mortality (k_x) during the pre-reproductive period was smaller (0.43) than that observed during the two reproductive peaks (1.27 and 0.6).

Physa acuta (N_0 = 208) also exhibited a concave survival curve (type III) (Table 2), but even more pronounced than for *Stenophysa marmorata*, because the highest d_x levels were recorded in the first week, with high values maintained until week 16 (Table 2). This species survived for 88 weeks. The mortality rate was quite high during the initial weeks, though a greater increment occurred between weeks 37 and 42 and later between weeks 72 and 80 (Table 2). The force of mortality during the pre- and post-reproductive periods was equal (0.3), but the value was lower than during the reproductive period (1.7). Although at most ages l_x values in *S. marmorata* were higher than in *P. acuta*, the latter species nevertheless had a greater longevity.

Both species laid eggs at night. *Stenophysa marmorata* began to oviposit during week 26 (at about six months), when l_x was 0.37, with a fecundity (m_x) of 0.09 (Table 1). Two reproductive efforts occurred, separated by two weeks. The first, during weeks 26 and 43, had a mean m_x value of 2.5 (*SD*: 1.8), but peaked at 6.7 at week 37. A pause in oviposition occurred, after 3 weeks coinciding with a high q_x. The second reproductive effort occurred over a shorter time period than the first (one month less) and attained a viability of only 0.99% of the original N_0, but exhibited greater mean m_x (10; *SD*: 7.5) and peaked at 31 during week 50.

Physa acuta began to lay eggs during week 13 with an m_x of 0.25 (Table 2) and an l_x of 0.49. This species reproduced continuously and reached the maximum of m_x (32.5) at week 69. After week 77 these snails had only one more oviposition, with but a single egg, at week 87 whose embryo failed to develop. Therefore, for this species the post-reproductive period extended for 11 weeks.

Although after each reproductive effort mortality rate increased in *Stenophysa marmorata*, this pattern was absent in *Physa acuta*. In *S. marmorata* those data were corroborated by a negative correlation between the values of m_x and q_x and between the values for m_x and k_x, corresponding to Spearman's coefficients of -0.554 (P = 0.001) and -0.517 (P = 0.002), respectively. These same variables were not significantly correlated in *P. acuta*.

The developmental period was similar for both species (Table 3), but with the mean number of eggs per oviposition being significantly lower in *Stenophysa marmorata* than in *Physa acuta* (Mann-Whitney test, U = 95021.5, Z = -15.6, $P <$ 0.0001). Significant differences were not observed in the number of ovipositions per individual (P = 0.329). Moreover,

Table 1. Horizontal life table of *Stenophysa marmorata*. Abbreviations: x, age in weeks; N_x1000, number of individuals at age x, expressed as a per thousand initial individuals (N_0); d_x, mortality; q_x, mortality rate; k_x, force of mortality; l_x, survival; m_x, fecundity; e_x, life expectancy; V_x, reproductive value at age x on the basis of the total number of eggs; V_xHE, reproductive value at age x on the basis of the number of hatched eggs.

x	N_x1000	d_x	q_x	k_x	l_x	m_x	e_x	V_x	V_xHE
0	1000	0	0	0	1		22.55	1.09	1.03
1	1000	4.95	0	0	1		21.55	1.17	1.06
2	995.05	0	0	0	1		20.65	1.26	1.10
3	995.05	4.95	0	0	1		19.65	1.35	1.13
4	990.10	39.60	0.04	0.02	0.99		18.75	1.45	1.17
5	950.50	29.70	0.03	0.01	0.95		18.48	1.62	1.25
6	920.79	4.95	0.01	0.00	0.92		18.05	1.79	1.33
7	915.84	4.95	0.01	0.00	0.92		17.14	1.92	1.37
8	910.89	29.70	0.03	0.01	0.91		16.23	2.07	1.42
9	881.19	39.60	0.04	0.02	0.88		15.74	2.29	1.51
10	841.58	34.65	0.04	0.02	0.84		15.44	2.57	1.62
11	806.93	99.01	0.12	0.06	0.81		15.06	2.87	1.74
12	707.92	59.41	0.08	0.04	0.71		16.02	3.51	2.04
13	648.51	4.95	0.01	0.00	0.65		16.40	4.10	2.29
14	643.56	14.85	0.02	0.01	0.64		15.52	4.42	2.37
15	628.71	143.56	0.23	0.11	0.63		14.86	4.85	2.50
16	485.15	4.95	0.01	0	0.49		17.96	6.73	3.33
17	480.20	0	0	0	0.48		17.13	7.28	3.46
18	480.20	24.75	0.05	0.02	0.48		16.13	7.79	3.56
19	455.45	54.46	0.12	0.06	0.46		15.96	8.80	3.86
20	400.99	0	0	0	0.40		16.99	10.70	4.51
21	400.99	0	0	0	0.40		15.99	11.46	4.63
22	400.99	19.80	0.05	0.02	0.40		14.99	12.27	4.77
23	381.19	4.95	0.01	0.01	0.38		14.71	13.83	5.16
24	376.24	4.95	0.01	0.01	0.38		13.89	15.00	5.37
25	371.29	0	0	0	0.37		13.07	16.28	5.60
26	371.29	14.85	0.04	0.02	0.37	0.09	12.07	17.43	5.76
27	356.44	4.95	0.01	0.01	0.36	1.53	11.53	19.34	6.17
28	351.49	24.75	0.07	0.03	0.35	0.75	10.68	19.34	5.80
29	326.73	4.95	0.02	0.01	0.33	1.48	10.41	21.42	5.79
30	321.78	4.95	0.02	0.01	0.32	3.11	9.55	21.68	5.65
31	316.83	14.85	0.05	0.02	0.32	2.94	8.69	20.19	5.16
32	301.98	4.95	0.02	0.01	0.30	1.84	8.07	19.39	4.96
33	297.03	39.60	0.13	0.06	0.30	2.02	7.18	19.11	4.84
34	257.43	9.90	0.04	0.02	0.26	1.46	7.13	21.12	5.45
35	247.52	14.85	0.06	0.03	0.25	2.36	6.38	21.89	5.77
36	232.67	24.75	0.11	0.05	0.23	7.06	5.72	22.25	6.16
37	207.92	4.95	0.02	0.01	0.21	5.14	5.29	18.20	5.86
38	202.97	14.85	0.07	0.03	0.20	2.51	4.39	14.32	5.25
39	188.12	4.95	0.03	0.01	0.19	3.13	3.66	13.65	5.01
40	183.17	103.96	0.57	0.36	0.18	5.86	2.73	11.56	4.49
41	79.21	29.70	0.38	0.20	0.08	4.25	4.00	14.11	6.82
42	49.50	29.70	0.60	0.40	0.05	1.10	4.80	16.90	11.22
43	19.80	0	0	0	0.02	4.00	9.50	42.30	28.85
44	19.80	0	0	0	0.02	0	8.50	41.01	29.67
45	19.80	9.90	0.50	0.30	0.02	0	7.50	43.92	30.51
46	9.90	0	0	0	0.01	4.50	13.00	94.06	62.76
47	9.90	0	0	0	0.01	9.00	12.00	95.90	64.54
48	9.90	0	0	0	0.01	11.00	11.00	93.06	66.38
49	9.90	0	0	0	0.01	5.50	10.00	87.87	64.66
50	9.90	0	0	0	0.01	31.00	9.00	88.21	61.87

Table 1. (Continued)

x	N_x1000	d_x	q_x	k_x	l_x	m_x	e_x	V_x	V_xHE
51	9.90	0	0	0	0.01	20.00	8.00	61.26	45.63
52	9.90	0	0	0	0.01	10.00	7.00	44.18	36.64
53	9.90	0	0	0	0.01	8.50	6.00	36.60	30.49
54	9.90	0	0	0	0.01	10.00	5.00	30.09	25.18
55	9.90	0	0	0	0.01	5.50	4.00	21.52	19.21
56	9.90	4.95	0.50	0.30	0.01	5.50	3.00	17.15	14.62
57	4.95	0	0	0	0	13.00	4.00	24.95	19.78
58	4.95	0	0	0	0	10.00	3.00	12.80	8.00
59	4.95	0	0	0	0	3.00	2.00	3.00	0
60	4.95	4.95	1	0	0	0	1.00	0	0

Table 2. Horizontal life table of *Physa acuta*. Abbreviations: x, age in weeks; N_x1000, number of individuals at age x, expressed as a per thousand initial individuals (N_0); d_x, mortality; q_x, mortality rate; k_x force of mortality; l_x, survival; m_x, fecundity; e_x, life expectancy; V_x, reproductive value at age x on the basis of the total number of eggs; V_xHE, reproductive value at age x on the basis of the number of hatched eggs.

x	N_x1000	d_x	q_x	k_x	l_x	m_x	e_x	V_x	V_xHE
0	1000	0	0	0	1		18.93	2.01	1.40
1	1000	129.81	0.13	0.06	1		17.93	2.21	1.50
2	870.19	43.27	0.05	0.02	0.87		19.45	2.79	1.86
3	826.92	76.92	0.09	0.04	0.83		19.42	3.23	2.11
4	750.00	52.88	0.07	0.03	0.75		20.31	3.91	2.50
5	697.11	14.42	0.02	0.01	0.70		20.77	4.62	2.89
6	682.69	24.04	0.03	0.02	0.68		20.19	5.18	3.18
7	658.65	38.46	0.06	0.03	0.66		19.89	5.90	3.55
8	620.19	0	0	0	0.62		20.06	6.88	4.06
9	620.19	14.42	0.02	0.01	0.62		19.06	7.56	4.37
10	605.77	19.23	0.03	0.01	0.61		18.49	8.50	4.81
11	586.54	67.31	0.11	0.05	0.59		18.07	9.64	5.35
12	519.23	24.04	0.05	0.02	0.52		19.28	12	6.50
13	495.19	72.11	0.15	0.07	0.49	0.25	19.16	13.8	7.34
14	423.08	19.23	0.04	0.02	0.42	0.32	21.26	17.4	9.25
15	403.85	38.46	0.09	0.04	0.40	0.21	21.23	19.6	10.42
16	365.39	33.65	0.09	0.04	0.37	0.71	22.36	23.58	12.32
17	331.73	0	0	0	0.33	1.00	23.52	27.67	14.50
18	331.73	0	0	0	0.33	0.23	22.52	29.30	15.39
19	331.73	0	0	0	0.33	0.90	21.52	31.93	16.47
20	331.73	9.62	0.03	0.01	0.33	2.13	20.52	34.08	17.35
21	322.12	4.81	0.02	0.01	0.32	2.31	20.10	36.14	18.50
22	317.31	4.81	0.02	0.01	0.32	3.14	19.39	37.72	19.22
23	312.50	0	0	0	0.31	2.46	18.68	38.58	19.33
24	312.50	4.81	0.02	0.01	0.31	3.86	17.68	39.67	19.85
25	307.69	9.62	0.03	0.01	0.31	3.69	16.94	39.95	19.50
26	298.08	4.81	0.02	0.01	0.30	4.53	16.45	41.11	19.81
27	293.27	0	0	0	0.29	4.89	15.71	40.84	20.15
28	293.27	4.81	0.02	0.01	0.29	3.98	14.71	39.49	20.17
29	288.46	24.04	0.08	0.04	0.29	4.20	13.93	39.65	20.46
30	264.42	4.81	0.02	0.01	0.26	4.04	14.11	42.48	22.21
31	259.62	0	0	0	0.26	8.59	13.35	43.01	22.61
32	259.62	0	0	0	0.26	7.13	12.35	37.81	15.09
33	259.62	9.62	0.04	0.02	0.26	6.59	11.35	33.70	13.67
34	250.00	24.04	0.10	0.04	0.25	7.96	10.75	30.92	12.76
35	225.96	24.04	0.11	0.05	0.23	6.19	10.79	27.90	11.83

Table 2. (Continued)

x	N_x1000	d_x	q_x	k_x	l_x	m_x	e_x	V_x	V_xHE
36	201.92	24.04	0.12	0.06	0.20	3.71	10.95	26.68	12.09
37	177.89	48.08	0.27	0.14	0.18	4.22	11.30	28.63	13.21
38	129.81	19.23	0.15	0.07	0.13	3.26	14.11	36.75	18.12
39	110.58	28.85	0.26	0.13	0.11	3.09	15.39	43.19	21.22
40	81.73	9.62	0.12	0.05	0.08	3.29	19.47	59.59	28.56
41	72.12	0	0	0	0.07	2.60	20.93	70.08	34.12
42	72.12	14.42	0.20	0.10	0.07	9.13	19.93	74.12	34.43
43	57.69	0	0	0	0.06	7.83	23.67	89.23	39.96
44	57.69	4.81	0.08	0.04	0.06	10.17	22.67	89.41	39.87
45	52.89	0	0	0	0.05	13.09	23.64	94.96	37.52
46	52.89	4.81	0.09	0.04	0.05	9.09	22.64	89.92	32.46
47	48.08	0	0	0	0.05	12.50	23.80	97.67	35.10
48	48.08	0	0	0	0.05	16.90	22.80	93.55	32.08
49	48.08	0	0	0	0.05	7.40	21.80	84.19	27.96
50	48.08	0	0	0	0.05	17.80	20.80	84.35	27.41
51	48.08	0	0	0	0.05	5.10	19.80	73.10	22.61
52	48.08	4.81	0.10	0.05	0.05	2.60	18.80	74.70	21.11
53	43.27	0	0	0	0.04	9.67	19.78	87.99	25.01
54	43.27	0	0	0	0.04	7.67	18.78	86.03	26.56
55	43.27	0	0	0	0.04	5.89	17.78	86.08	28.23
56	43.27	0	0	0	0.04	3.78	16.78	88.08	29.19
57	43.27	0	0	0	0.04	13.56	15.78	92.60	29.86
58	43.27	0	0	0	0.04	15.33	14.78	86.83	30.47
59	43.27	0	0	0	0.04	24.33	13.78	78.53	22.63
60	43.27	0	0	0	0.04	10.00	12.78	59.53	12.88
61	43.27	4.81	0.11	0.05	0.04	2.89	11.78	54.40	10.52
62	38.46	4.81	0.13	0.06	0.04	1.50	12.13	63.66	12.60
63	33.65	4.81	0.14	0.07	0.03	8.86	12.71	78.03	15.50
64	28.85	0	0	0	0.03	16.33	13.67	88.64	19.29
65	28.85	0	0	0	0.03	4.33	12.67	79.43	17.53
66	28.85	0	0	0	0.03	8.50	11.67	82.48	17.62
67	28.85	0	0	0	0.03	7.83	10.67	81.26	18.96
68	28.85	0	0	0	0.03	11.33	9.67	80.66	20.05
69	28.85	0	0	0	0.03	0	8.67	76.15	15.13
70	28.85	0	0	0	0.03	32.50	7.67	83.64	16.28
71	28.85	4.81	0.17	0.08	0.03	23.17	6.67	56.18	8.73
72	24.04	9.62	0.40	0.22	0.02	22.20	6.80	43.51	5.47
73	14.42	0	0	0	0.01	22.67	9.67	39.01	4.07
74	14.42	0	0	0	0.01	0.67	8.67	17.95	1.87
75	14.42	0	0	0	0.01	7.00	7.67	18.99	2.01
76	14.42	0	0	0	0.01	0	6.67	13.17	2.17
77	14.42	4.81	0.33	0.18	0.01	14.33	5.67	14.46	2.33
78	9.62	0	0	0	0.01	0	7.00	0.22	0
79	9.62	0	0	0	0.01	0	6.00	0.24	0
80	9.62	4.81	0.50	0.30	0.01	0	5.00	0.26	0
81	4.81	0	0	0	0.01	0	8.00	0.57	0
82	4.81	0	0	0	0.01	0	7.00	0.63	0
83	4.81	0	0	0	0.01	0	6.00	0.69	0
84	4.81	0	0	0	0.01	0	5.00	0.76	0
85	4.81	0	0	0	0.01	0	4.00	0.83	0
86	4.81	0	0	0	0.01	0	3.00	0.91	0
87	4.81	0	0	0	0.01	1.00	2.00	1.00	0
88	4.81	4.81	1.00	0	0.01	0	1.00	0	0

Table 3. Characterization of the ovipositions by *Stenophysa marmorata* and *Physa acuta*.

Attributes	*S. marmorata*	*P. acuta*
Total number of ovipositions	466	855
Total number of eggs	2,434	8,203
Mean (*SD*) number of eggs per oviposition	5.17 (2.37)	9.58 (6.35)
Maximum number of eggs per oviposition	17	50
Minimum number of eggs per oviposition	1	1
Mean (*SD*) time of embryonic development (days)	17 (7.32)	14 (7)

the proportion of hatched eggs was greater in *P. acuta* than in *S. marmorata* (Contingency table, Chi-square = 135.2, $P < 0.0001$). In the latter species, fewer than one-third of the eggs hatched, and more than one-half of the embryos were not developed (Fig. 1). In addition, 103 empty eggs were recorded; two being with two embryos, with one of those embryos having a lethal cephalic malformation and the other hatching without a shell. In *P. acuta*, two eggs without an embryo and another two with two embryos were observed. Nearly half of the eggs hatched, whereas one-third failed to continue development (Fig. 1). Two embryos exhibited cephalic malformations and did not hatch, while another was born without a shell.

Physa acuta had significantly higher values of m_x compared to *Stenophysa marmorata*, on the basis of either the total number of eggs per individual (Mann-Whitney test, $U = 926.5$, $Z = -1.5$, $P = 0.063$) or hatched eggs per individual (Mann-Whitney test, $U = 953$, $Z = -1.3$, $P = 0.091$). In addition, *P. acuta* had a more-lengthy reproductive period. The total number of eggs laid by individuals during the entire reproductive period, calculated as the sum of the m_x values, was only about 200 in *S. marmorata*, but over 480 in *P. acuta*.

In *Physa acuta*, the mean number of eggs per oviposition increased with the snails's age, and a significant correlation existed (Fig. 2). This relationship was not observed in *Stenophysa marmorata* (Fig. 2). In both species, however, the total number of eggs and ovipositions per specimen increased with the gastropods' age, with that correlation being greater in *S. marmorata* (Figs. 3, 4). On the basis of only the hatched eggs per individual, a positive correlation with age was observed in *S. marmorata*, whereas this relationship did not

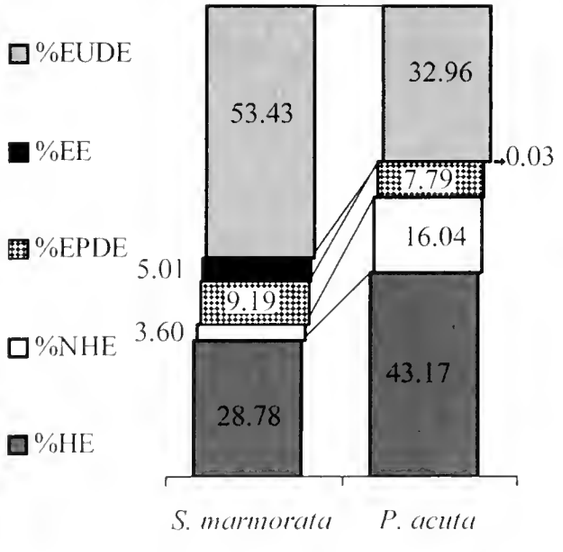

Figure 1. Percentage of hatched eggs (HE), non-hatched eggs with complete and normally developed embryos (NHE), eggs with partially developed embryos (EPDE), eggs with undeveloped embryos (EUDE), and empty eggs (EE) among the total eggs laid by *Stenophysa marmorata* and *Physa acuta*.

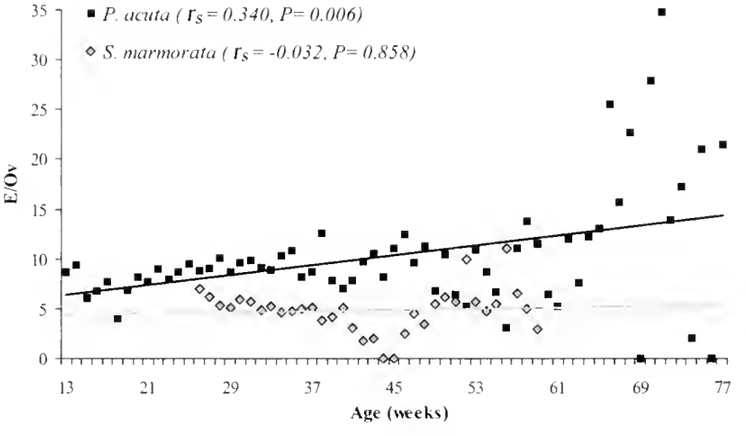

Figure 2. Mean number of eggs per oviposition (E/Ov) per age in *S. marmorata* ($N = 34$) and in *P. acuta* ($N = 65$). r_s, Spearman's Correlation Coefficient.

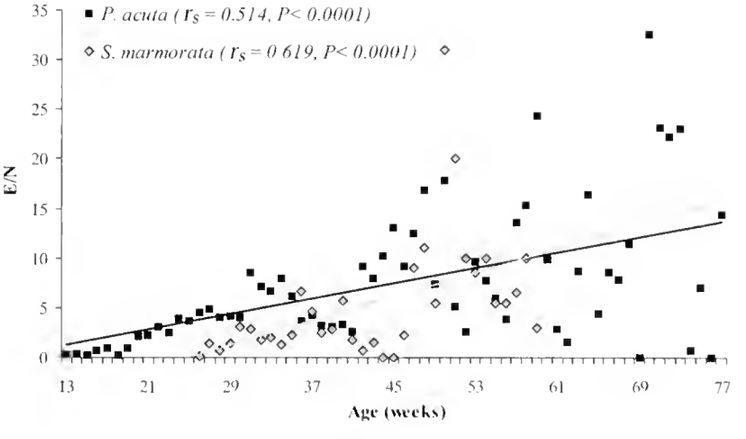

Figure 3. Mean number of eggs laid per individual (E/N) at each age in *S. marmorata* ($N = 34$) and in *P. acuta* ($N = 65$). r_s, Spearman's Correlation Coefficient.

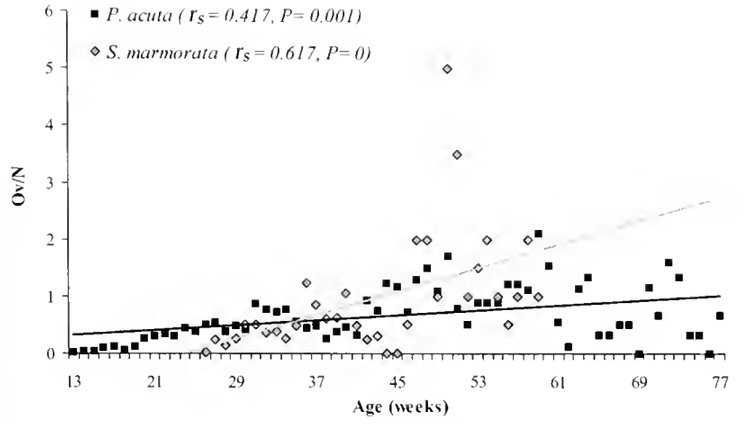

Figure 4. Mean number of ovipositions per individual (Ov/N) at each age in *S. marmorata* ($N = 34$) and in *P. acuta* ($N = 65$). r_s, Spearman's Correlation Coefficient.

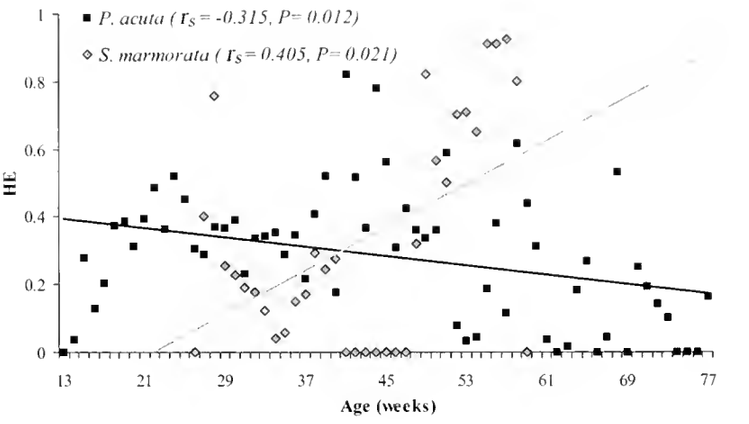

Figure 6. Mean of hatched eggs (HE) at each age in *S. marmorata* ($N = 34$) and in *P. acuta* ($N = 65$). r_s, Spearman's Correlation Coefficient.

occur in *P. acuta* (Fig. 5). This difference resulted because in *S. marmorata* the proportion of hatched eggs tended to increase with age, but in *P. acuta* this parameter showed an age-associated decrease (Fig. 6).

Life expectancy (e_x) exhibited a similar profile with age in both species, but with consistently higher values for *Physa acuta* (Chi-square $\chi^2 = 247.1$, $df = 59$). In both species a decrease occurred from week 22 on, but during approximately the fortieth week a new increase began (Tables 1-2). In *Stenophysa marmorata*, e_x values peaked at 13 in week 46, but was not greater than during the first weeks (with a maximum of 22.6 in week 1). In contrast, *P. acuta* reached its maximum e_x of 23.8 at week 47.

In *Stenophysa marmorata*, V_x increased between weeks 43 and 54, ranging from 30.1 to 95.9 (Table 1). Individuals of those ages, therefore, make a higher contribution to the next generation. In *Physa acuta* high values of V_x occurred for a more extensive length of time, between weeks 39 and 72, with values between 43.2 and 97.7 (Table 2).

Moreover, the mean number of offspring produced per individual of *Stenophysa marmorata* throughout its entire life was 12.1 (the replacement rate, or R_0), less than one-third of that in *Physa acuta*, 39.4. This difference was highly significant ($P < 0.0001$).

When only hatched eggs were considered, in *Stenophysa marmorata*, the m_x value diminished, while the period without viable eggs (between the two reproductive efforts) extended from 2 to 7 weeks. In addition, the values of V_x decreased (Table 1) and especially those of R_0, which dropped to 2.93, only one-fourth of its original value. In *Physa acuta* m_x, V_x, and R_0 likewise declined, but less than in *S. marmorata* (Table 2); while the net reproductive rate decreased by nearly one-half (15.0), it still remained fivefold higher than the observed value in *S. marmorata*.

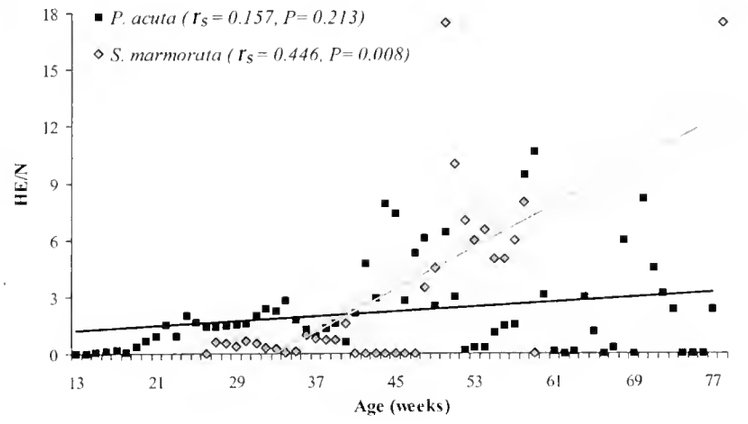

Figure 5. Mean number of hatched eggs per individual (HE/N) at each age in *S. marmorata* ($N = 34$) and in *P. acuta* ($N = 65$). r_s, Spearman's Correlation Coefficient.

DISCUSSION

Results obtained in the laboratory indicate a more successful reproductive strategy in the exotic snail, as compared to the native, corroborating field observations, where *Stenophysa marmorata* exhibits a markedly seasonal reproductive cycle. *Physa acuta*, in contrast, reproduces during the entire year and at these latitudes is not limited temperatures cold enough to freeze the water, as in the Northern Hemisphere, and begins to reproduce at a smaller size (Núñez 2009).

The pre-reproductive period of *Physa acuta* was only half as long as that of *Stenophysa marmorata*. Although the length of this interval depends on the temperature (Thomas and McClintock 1990), in this circumstance temperature conditions

were the same for both species. In contrast to Clampitt (1970), the more extended reproductive period and lower individual growth rate of *P. acuta*, as compared to *S. marmorata* (Núñez 2009), does not indicate that the former requires more stable environments; on the contrary, *P. acuta* is found in a wider variety of environments.

Both *Physa acuta* and *Stenophysa marmorata* are able to inhabit semi-permanent habitats although neither can survive in the absence of water (Núñez 2009). Nevertheless, the earlier sexual maturation of *P. acuta* enables it to either repopulate or colonize other environments more easily than *S. marmorata*. Moreover, the laboratory results here, with respect to greater fecundity values and higher percentages of viable eggs, apart from that more rapid maturation, corroborate the existence of reproductive characteristics giving a distinct colonization and survival advantage over the latter species.

In the field, I observed competition for space that has resulted in reduction in both the density and the area of occupation of *Stenophysa marmorata* although up until the present no competitive exclusion has occurred (Núñez 2009). The same form of exclusion could presumably occur with *Biomphalaria peregrina* (d'Orbigny, 1835) (Planorbidae). In artificial lakes of recent origin in the north of the Buenos Aires Province, *Physa acuta* was among the first species colonizing those environments, together with the native *Pomacea canaliculata* (Lamarck, 1822) (Ampullariidae), *Heleobia parchappii* (d'Orbigny, 1835) (Cochliopidae), and more belatedly, *B. peregrina* (Núñez 2009). The first of these species was located at greater depths, and the second over substrate in shallow water, whereas *B. peregrina* colonized submerged vegetation, where *P. acuta* was also present. However, *B. peregrina* never attained the same densities as the other three species. Under laboratory conditions, it began reproduction later than *P. acuta* (at week 17), had a shorter reproductive period (30 weeks), and had lower fecundity values (Rumi 1993). The appearance of *P. acuta* in limnobiotopes within Argentina thus apparently reduces species richness and diversity through altering relative abundances of the native gastropods.

According to Rankin and Harrison (1979), *Stenophysa marmorata* is an r-selected, pioneering species capable of rapid exploitation of favorable conditions, but this study indicates that those characteristics are more pronounced in *Physa acuta*.

My results are in accord with the characteristics proposed for *Physa acuta* by Dillon and Wethington (2004), *e.g.*, early sexual maturation and high reproductive rates. According to the USR model of life history variation in freshwater molluscs, populations are classified in U-populations, Undifferentiated with respect to reproductive effort, S-adapted, for Stress-tolerant, and R-adapted, for Reproductive recklessness (Dillon 2000). Both of these species could be classified as R-strategists although *Stenophysa marmorata* devolves more energy to growth at the cost of later maturation and longevity in comparison to *P. acuta*, and the latter is iteroparous. On the basis of all these characteristics, *P. acuta* has reproductive characteristics typical of a highly invasive species (Morton 1996).

Dillon and Wethington (2004) demonstrated that intraspecific variation exists in the life histories of different populations and attributed this variability to the diversity and spatial isolation of the habitats from which those populations arose. Even though in this instance the two snail populations used in these laboratory experiments were separated by less than 7' in latitude and 24' in longitude, the differences were still statistically significant.

Despite the similarity that the two species exhibited in the distribution of the mortality, *Stenophysa marmorata* survived for a much shorter time because reproduction affected survival. Both of these snails suffered high mortality during initial weeks; nevertheless, both the mortality rate and the force proved greater around week 40, and neither survival curve was as markedly concave as the curves previously observed for *Biomphalaria peregrina*, likewise under laboratory conditions (Rumi 1993).

Although laboratory experiments necessarily underestimate mortality, the similar conditions allow comparison of populational parameters (*i.e.*, life expectancy, reproductive value, and net reproductive rate) which indicate populational-growth potential is demonstrably greater in the exotic species.

Therefore, the high worldwide distribution as well as the omnipresence of the exotic *Physa acuta* with respect to the native *Stenophysa marmorata* in Argentina could be explained, at least in part, by early sexual maturation, higher reproductive potential, and greater longevity. The importance of interspecific competition between these two species needs, however, to be the subject of further study.

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Clarifying the northern distributional limits of the slipper limpet *Crepidula fornicata* in the northwestern Atlantic

Timothy A. Rawlings[1], Jana M. Aker[1], and Pierre Brunel[2]

[1]Department of Biology, Cape Breton University, 1250 Grand Lake Road, Sydney, Nova Scotia, B1P 6L2, Canada

[2]Institut Québécois de la Biodiversité (IQBIO), Département de Sciences Biologiques, Université de Montréal, Montréal, Québec, H3C 3J7, Canada

Correspondence, T. A. Rawlings: Timothy_Rawlings@cbu.ca

Abstract: The distribution of the slipper limpet *Crepidula fornicata* (Linnaeus, 1758) in the northwestern Atlantic is generally understood to extend only as far north as Baie des Chaleurs (~48°N, 66°W) in the southwestern Gulf of St. Lawrence and only as far east as Cape Breton Island, Nova Scotia (~46°N, 60°W). Here we present evidence that this species is found considerably further north (to 51°N) and east (to 55°W) than previously recognized. More specifically, its distribution is now known to include the Quebec "Lower North Shore" of the Gulf of St. Lawrence as well as the southern and western coasts of Newfoundland. Our evidence is based upon (1) shell collections from 12 museums in Canada and the U.S., (2) reports from both the primary and the secondary literature dating back to 1841, and (3) collections of *C. fornicata* in Newfoundland in 2009. While our investigations suggest that *C. fornicata* is expanding its geographic range in eastern Canada, we cannot be certain of this since the northern Gulf of St. Lawrence, Newfoundland, and Labrador have not been extensively sampled in the past for non-commercial benthic marine molluscs. Factors such as larval drift in known surface currents may be sufficient to explain such supposed range extensions. Clarification of current geographic ranges of marine species, particularly of well-studied and easily recognizable taxa such as *C. fornicata*, remains an important task for establishing temporal benchmarks against which to assess the effect of climate change on the marine environment.

Key words: geographic range, gastropod, climate change

Climate change can impact marine ecosystems through contractions and expansions of species' geographic ranges (Southward *et al.* 1995, Jackson 2008, Cheung *et al.* 2009). Predicting the response of an organism to such environmental changes requires a good understanding of its present geographic range, its ecology, and its tolerance for temperature, salinity, oxygen levels, and turbidity (Derivera *et al.* 2007, Therriault and Herborg 2008, Cheung *et al.* 2009). Even for charismatic or well-studied marine taxa, making such predictions can be challenging given that current geographic ranges are often not well circumscribed. Assessing distributional limits can be particularly difficult for broadly dispersed taxa for which reference specimens are not well represented across their geographic range, and for which literature from past collection efforts is not widely accessible.

The slipper limpet *Crepidula fornicata* (Linnaeus, 1758) is an easily identified and widely-studied marine species with a poorly defined native geographic distribution. These snails are common in shallow subtidal (<50 m) marine habitats along the eastern seaboard of the United States, where their presence is often revealed by their characteristic "slipper-like" shells washing onshore. The geographic distribution of this species in North America is extremely broad: Malacolog, a database of western Atlantic marine molluscs (Rosenberg 2009), lists a latitudinal range of 25°N to 48°N for *C. fornicata*, with its northern range extending into three Canadian provinces, New Brunswick (NB), Prince Edward Island (PEI), and Nova Scotia (NS). Publications focusing on *Crepidula* have presented northern geographic range limits in the northwestern Atlantic associated with the southern Gulf of St. Lawrence, and more specifically PEI (Hoagland 1977, Collin 2001) or NB (Escuminac Point, NB; Blanchard 1997). These northern distributional limits are not well supported, however, and conflict, to varying degrees, with regional publications based upon faunal inventories of the Gulf of St. Lawrence (*e.g.*, Brunel *et al.* 1998). To clarify the current geographic range of this species in eastern Canada, here we document (1) distributional records published before 1934 in the widely-available English literature, (2) more recent distributional records in regionally-available literature, including those by Quebec (QC) investigators, (3) unpublished museum records from 12 museums in Canada and the U.S., and (4) the results of recent shallow-water collections of this species.

MATERIALS AND METHODS

Literature review

Our literature review began in 1989 with a species-level catalogue of all marine invertebrates recorded from the Gulf

of St. Lawrence, funded by the St. Lawrence Vision 2000 program and the Maurice Lamontagne Institute (Fisheries and Oceans Canada) in Mont-Joli, QC (Brunel *et al.* 1998). Reviews of the early literature were provided by Ganong (1887) and Whiteaves (1901) as well as earlier (from 1956) private communication with east coast scientists (*e.g.*, A. G. Huntsman, E. L. Bousfield). The systematic retrieval of new articles was subsequently achieved through accessing the *Zoological Record* back to 1902. In addition, reports and papers from the grey literature were actively searched for, especially those by authors associated with the Fisheries Research Board of Canada and its earlier designations. Papers published by the Quebec Department of Fisheries and the equivalent services in the four Atlantic provinces bordering the Gulf of St. Lawrence, including the pre-1949 Newfoundland (NL) government, were also examined. Promising titles cited in those publications were further investigated, with an emphasis on regional studies in the Gulf of St. Lawrence. Private (*e.g.*, P. Brunel, E. Bourget) and public collections (*e.g.*, Maurice Lamontagne Institute) were also used. Monographs and field guides covering the western Atlantic and the Arctic were searched for general information on geographic distributions. Only the most relevant field guides were retained in our review. The literature review for *Crepidula fornicata* and other marine invertebrate taxa of the Gulf of St. Lawrence was reported in Brunel *et al.* (1998). In 2009, we verified these older distributional records for *C. fornicata*, adding to those presented in Brunel *et al.* (1998). We also searched for recent primary research articles through Web of Science literature reviews using key words or papers citing seminal publications on *Crepidula*, such as Hoagland (1977).

Surveys of museum records, shorelines, and shallow marine habitats

We employed two additional methods to ascertain the northern range of *Crepidula fornicata* in the northwestern Atlantic. First, we examined collections from major museums and reference collections in northeastern North America, including the Academy of Natural Sciences of Philadelphia (ANSP), the American Museum of Natural History (AMNH), the Atlantic Reference Centre (ARC), the Canadian Museum of Nature (CMN), the Field Museum of Natural History (FMNH), the Maurice Lamontagne Institute (MLI), the Museum of Comparative Zoology, Harvard (MCZ), the National Museum of Natural History (USNM/NMNH), the Nova Scotia Museum of Natural History (NSMNH), the Peabody Museum of Natural History (PMNH), the Royal Ontario Museum (ROM), and The Rooms Provincial Museum (Newfoundland and Labrador; NFM). Distributional information from these 12 collections was based on inventories provided by collections' staff. For incomplete museum records from critical parts of *C. fornicata*'s range, we also obtained as much missing data as possible, especially geographic coordinates and dates of sampling. We verified databases, either personally or with the assistance of colleagues, by direct examination of specimens from some of these collections (CMN, MLI, NFM, ROM); in other cases we relied on the correct identification and record keeping of the curatorial staff. Our verification of collection information for *C. fornicata* demonstrated a high degree of reliability, likely in part because this is a well known species, but also because there are only two other species of *Crepidula* reported from eastern Canada: *C. plana* Say, 1822 and *C. convexa* Say, 1822 (Brunel *et al.* 1998, Rosenberg 2009). *Crepidula plana* is abundant, but has distinctive flat, white shells unlikely to be confused with *C. fornicata*. *Crepidula convexa*, which can be confused with small *C. fornicata*, appears to be rare within eastern Canada. Only two museum lots of this species were uncovered in our survey of the Gulf of St. Lawrence (USNM 224957; MCZ 144822); in addition, only two research publications, Verrill (1873) and Medcof and Morrison (1943), directly support its presence within this geographic region.

Second, we made new collections of *Crepidula fornicata* in 2009 and 2010. Based on literature and museum records of *C. fornicata* from NL, we explored shorelines and shallow marine habitats close to its northern distributional limits in western NL in July 2009. Sampling involved beach walks and/or snorkel surveys in the region of St. George's Bay, Port au Port Bay, Bonne Bay, and St. Paul's Inlet, in western NL. No recent fieldwork was conducted on the south coast of NL. In addition, to investigate distributional limits in the southwestern Gulf of St. Lawrence, in July 2010 we investigated the northern shore of the Baie des Chaleurs between Carleton and Pasbébiac, east of New Carlisle. Select beaches were surveyed for shells of *C. fornicata* through hour-long beach walks encompassing both the lower and higher regions of the shoreline.

RESULTS

Pre-1934 literature survey

The northern distribution of the slipper limpet has long been known to correspond with the so-called Virginian or warm-temperate fauna, which mainly ranges south of Cape Cod (Ganong 1887, 1890), but reappears north in the southwestern Gulf of St. Lawrence, the "Magdalen Shallows" enclave. The American oyster *Crassostrea virginica* (Gmelin, 1791) is the best known representative of this fauna, and *Crepidula fornicata* commonly attaches to oyster shells and other hard substrates (Galtsoff 1964).

The pre-1934 distributional records for *Crepidula fornicata* in eastern Canada are listed in chronological order in Table 1, beginning with Lyell (1841) who first noted the presence of *C. fornicata* in the Gulf of St. Lawrence. The

Table 1. Chronology of historical references (1841-1934) on the distribution of *Crepidula fornicata* in the Gulf of St. Lawrence, updated from Brunel *et al.* (1998).

Authors	Year	Original or translated quotations on localities and (notes)
Lyell	1841	"Gulf of St. Lawrence" (p. 138; without localities)
Bell	1859a	"One specimen found at Dalhousie, NB, very abundant at Caraquette" (p. 212; first locality on southwestern shore, second one on southeastern shore of Baie des Chaleurs)
Bell	1859b	"Dalhousie, Bay of Chaleur" (p. 254)
Gould and Binney	1870	"...on oysters from PEI, off the mouth of the St. Lawrence" (p. 271)
Dawson and Harrington	1871	"no dredging" (p. 50; only inshore sampling in Northumberland Strait)
Verrill *et al.*	1873	"...southern part of the Gulf of Saint Lawrence, PEI, &c" (pp. 649-650)
Whiteaves	1874a	"Between Cape Bear and Pictou Island" (p. 217) and "on oyster beds of Shediac Bay, N.B." (p. 217) (both in southeastern Northumberland Strait)
Whiteaves	1874b	"from the area between Prince Edward Island and Cape Breton, through Northumberland Straits, along the coast of New Brunswick as far to the north as the southern entrance to the Bay des Chaleurs; Carraquette Bay, N.B. seems to be their extreme northern limit." (p. 189) and cited in "...list of species collected in Shediac Bay" (p. 199)
Verrill	1879	(Listed for the area between Cape Cod and Gulf of St. Lawrence)
Bain	1885	(Recorded from PEI, without localities)
Whiteaves	1886	"Pictou, NS" (p. 28; southeastern Northumberland Strait)
Ganong	1887	"Dalhousie, Caraquette, (very abundant), Bell (C). Bay Chaleur, Whiteaves (D), Shediac, Whiteaves (I). Northumberland Straits and along the coast to Caraquette Bay, Whiteaves (H). Not found on the southern coast" (p. 38; letters between parentheses refer to bibliographic sources listed.)
Winkley	1888	"mostly from Summerside, PEI" (p. 69); "occurs in thousands" (p. 70)
Ganong	1890	(Species listed in table on p.172, under column "Gulf of St. Lawrence")
Provancher	1890	(Listed from the Province of Quebec, without localities)
Provancher	1891	"in southern part of Gulf of St. Lawrence, PEI, attached to various shells and especially oysters" (p. 88)
Winkley	1892	Among "colony of warm water shells in the waters of Northumberland Strait" (p. 63)
Conklin	1897	"...from Labrador to Florida" (p. 5; without localities)
Whiteaves	1901	"Abundant on oysters throughout the whole oyster region north-ward to Caraquette Bay" and "...in Northumberland Strait" (p. 168)
Chadwick	1906	"Bedeque Bay, common" (p. 103; PEI)
Halkett	1907	"Pictou, NS" (p. 347)
Ives	1907	"...quite common throughout the whole oyster region", "...not plentiful outside of low water mark" (typewritten copy seen only, with changed pagination; no specific reference to Northumberland Strait)
Stafford	1912a	"Malpeque" (p. 41, in checklist of species)
Stafford	1912b	"Canso, Malpeque" (p. 77; summary of earlier report)
Jones	1924	(from two stations) "Shippigan Gully... and Sound" (p. 2 + 16, northeastern NB, Baie des Chaleurs)
Dall	1926	"mouth of Burnt Church River, north side of Miramichi Bay, NB" (p. 153; none found in collections made in western NL)
Dall	1929	"Escuminac Point", NB (p. 159); "Buctouche", "beach at Cocagne Harbor", "beach at Grant (between Shemagoe Inlet and Cape Tormentine)", NB (p. 160)
Johnson	1934	"PEI to Texas and West Indies" (p. 97)

earliest detailed record of this species in the Gulf was provided by Bell (1859a), whose two localities placed the northernmost limit of *C. fornicata* along the southern shore of Baie des Chaleurs in NB, a region warmer than the northeastern shore of this bay in QC (Jones 1924, Tremblay 1944, Brunel 1959, Legendre and Watt 1970). Most subsequent records, including Ganong (1887), a reliable source for other marine molluscs along the NB coast, confirmed this species as abundant and

typical of the Magdalen Shallows, mostly around PEI and the Magdalen Islands. One exception to this pattern was Conklin (1897), who listed the geographic range of *Crepidula* to include "Labrador". Labrador at that time referred to a large part of the Quebec-Labrador peninsula, including the eastern reaches of the north shore of the Gulf of St. Lawrence—a region known locally as the "Lower North Shore" (J. Maunder, pers. comm.). The basis for Conklin's inclusion of Labrador in the range of *C. fornicata* remains uncertain since this record was not supported by additional information nor by the earlier and thorough survey of Packard (1863, 1867).

Post-1934 publications

Little was published in the primary literature on the distribution of *Crepidula fornicata* in eastern Canada between 1934 and 1950. This changed significantly in the 1950s with the hiring of zoologist Edward L. Bousfield at the National Museum of Canada (NMC). Bousfield's explorations of the intertidal fauna of the Canadian Maritime Provinces (Bousfield 1952, 1955a, Bousfield and Laubitz 1972) quickly added to the research collections of the NMC (now the Canadian Museum of Nature) with publications on the distributional ranges of a broad set of taxonomic groups, including molluscs. Since 1950, of the 46 publications listing the distribution of *C. fornicata* in eastern Canada (Table 2), only 22 were based upon actual sampling: the remaining ones contained secondary information of variable quality on the distribution of this species or simply repeated previous records by the same author. A majority of the new sources—16 out of 22—merely confirmed historical records that the distribution in the Gulf of St. Lawrence encompassed the area around PEI northward to the Miramichi Estuary as well as the Magdalen Islands (Table 1). Included in this, Bousfield (1952, 1955a, 1960) provided general information on the distribution which he assumed to fit into that of the Virginian "warm-water fauna" already known since Ganong (1887, 1890), but he listed no localities to support his claim.

Significant primary records during this period relevant to the distributional limits of *Crepidula fornicata* reported the species from Baie des Chaleurs (Corbeil 1953, Logie 1953, Ledoyer 1975, Schafer and Wagner 1978) as well as from new localities in western NL (Mercer 1970, Hooper 1975, Carter and McGregor 1979), and the Quebec Lower North Shore (D'Amours and Pilote 1982). Among secondary publications, only La Rocque (1953), Bousfield (1960), Brunel (1970a), Dunbar *et al.* (1980), and Brunel *et al.* (1998) correctly cited the known historical northern limit of *C. fornicata*'s distribution in Baie des Chaleurs (~48°N). In 1955, Bousfield (1955a) suggested that *C. fornicata* might be found as far to the northeast as Gaspé Bay (north of Baie des Chaleurs) where other Virginian species can be found, but in his 1960 publication limited their northern distribution to "Chaleur Bay". This modification was later supported by the absence of this species in Gaspé Bay despite extensive sampling by Brunel (1970b and unpub. data) and Ledoyer (1975). Recent publications focusing on northern Atlantic *Crepidula* have provided vague and somewhat misleading northern geographic range limits for *C. fornicata*, with the northernmost latitudinal boundaries associated with PEI (Hoagland 1977, Collin 2001) or NB (Escuminac Point, NB; Blanchard 1997). Collin (2003) categorized *C. fornicata*'s northern range as extending >50°N, presumably because of the higher latitudes associated with its introduced range in Europe (59°N, Blanchard 1997) rather than its long-known native geographic range reaching Baie des Chaleurs (Bell 1859a, 1859b, Whiteaves 1901) in the northwestern Atlantic. Only one record, cited in Bourget (1997), is known to be erroneous (Bourget, pers. comm.): recent *C. fornicata* specimens have never been taken in the St. Lawrence Estuary, despite intensive inshore and offshore collecting since 1929 (Brunel *et al.* 1998).

The earliest publication to suggest the presence of *Crepidula fornicata* along NL's coastline was Bousfield's (1960) field guide which included "southwestern Newfoundland" within the range of this species (Table 2). Because Bousfield's 1960 range extension did not cite additional information or reference museum specimens, this northern locality went unnoticed or unaccepted by many subsequent workers (*e.g.*, Hoagland 1977, Collin 2001). In 1968, Mercer (1970) also recorded *C. fornicata,* along with several other epibionts, growing on oysters introduced to Two Guts Pond (Port au Port Bay) as seed and adults from PEI in 1965. In 1975, Bousfield and Thomas described *C. fornicata* as a component of the "very eurytopic warm water species" of the Canadian Atlantic "in all warm-water areas, including the west coast of Newfoundland..." (p. 57), but failing to reach the St. Lawrence Estuary. *Crepidula fornicata* was first reported to the north in Lomond, Bonne Bay (49°27'N, 57°45'W) and St. Paul's Inlet (49°51'N, 57°47'W) by Hooper (1975) as part of an ecological and biological assessment of Bonne Bay (and surrounding regions) for the Canadian National Parks Service in the early 1970s. Carter and MacGregor (1979), in their survey of St. Paul's Inlet, documented the location of *C. fornicata* within the eastern arm of that inlet, based upon surveys in 1977-78. These latter reports and other grey literature focusing on the benthic marine molluscs of Newfoundland and Labrador were brought to wider attention by Gilkinson (1986).

Brunel *et al.* (1998) reported *Crepidula fornicata* along the northeastern QC coastline of the Gulf of St. Lawrence, within the region defined above as the Lower North Shore. This record was based upon an inshore scallop survey in August 1981 in which 200 dredge samples were taken across

Table 2. Chronology of recent references (1950-2009) on the distribution of *Crepidula fornicata* in the Gulf of St. Lawrence, updated from Brunel *et al.* (1998).

Authors	Year	Original or translated quotations on localities and (notes)
Miner	1950	"...from PEI to Texas..." (p. 629)
Bousfield	1952	"The beaches of the gulf coast of NS, NB, and PEI present an entirely different picture."... "Species very commonly encountered are...the slipper limpets (*Crepidula* spp.)..." (p. 191)
Corbeil	1953	(recorded from scallop survey westward from Carleton, QC, northwestern shore of Baie des Chaleurs, and Black Point, NB)
La Rocque	1953	"Chaleur Bay to Texas and West Indies" (p. 160)
Logie	1953	"at Shippigan this year the slipper limpet constituted an additional fouling organism for the first time in the writer's experience. " (p. 16)
Warburton	1953	"Malpeque Bay, PEI....Limpets of two species, *Crepidula fornicata* and *C. plana* often occur on such shells" (p. 21; *i.e.*, "*Cliona*-inhabited oyster shells")
Abbott	1954	"Canada to Florida and to Texas" (p. 170)
Bousfield	1955a	"The fourth subregion takes in the entire north shore of Chaleur Bay eastward to and including Gaspe Bay" and "Many southern marine species reach their northern limit of distribution here, viz the gastropods *Crepidula fornicata*..." (p. 100)
Walne	1956	"...from Escuminac Point, New Brunswick, (47°N.), and Summerside, Prince Edward Island, in Canada, to the Caribbean islands..." (p. 1)
Bousfield	1960	"Chaleur Bay to southwestern NL and NS" (p. 13)
Carbonneau	1967	"caught relatively frequently" (p. 22; in scallop survey off the Magdalen Islands, QC)
Brunel	1970a	(Listed on p. 21 from subtidal depths in Baie des Chaleurs and the Magdalen Islands, QC)
Mercer	1970	On oysters, "Two-Guts Pond", Port au Port (p. 25; southwestern NL)
Thomas	1970	"Smelt Creek (p. 2), Bideford Estuary" (p. 6 + 17; northwestern PEI)
Hughes and Thomas	1971a	(Appendices IIa-c: recorded on Paugh's Creek transect (6/15 stations), Fred England Bed transect (8/17 stations), Martin Bed transect (9/21 stations) and Totten Bed transect (42/51 stations), Bideford River Estuary, PEI)
Hughes and Thomas	1971b	Bedeque Bay (PEI)
Gosner	1973	"BV" (*i.e.*, "boreal, from Cape Cod to Labrador" + "Virginian, from Cape Cod to Cape Hatteras" (p. 263, somewhat updated from Johnson, 1934)
Abbott	1974	"Canada to Florida and to Texas"(p. 141)
Hooper	1975	"Lomond, Bonne Bay" and "St. Paul's Inlet" (p. 140; western NL)
Ledoyer	1975	(dredge haul off Miscou and Shippigan Islands, northeastern NB)
Wagner	1975	Strait of Canso (NS, southeastern tip of Gulf of St. Lawrence; full range of species given as 12°N to 48°N in table 6, p. 13)
Wagner	1976	Miramichi Estuary (northeastern NB)
Caddy *et al.*	1977	Northumberland Strait and Georgetown Bay, PEI (in table 4, p. 15)
Hoagland	1977	"...north to PEI" (p. 375; based on collections examined at major museums in the U.S., England and Germany)
Schafer and Wagner	1978	Eastern Baie des Chaleurs
Carter and McGregor	1979	St. Paul's Inlet (western NL) (map on p. 136)
Gosner	1979	"Gulf of Mexico north to Massachusetts Bay, locally to Gulf of St. Lawrence, in lower intertidal zone. Accidental in Europe." "...especially along protected bays and sounds." (p. 129)
Dunbar *et al.*	1980	(Dots on map: south of Magdalen Islands, Northumberland Strait and Caraquet area, southeastern shore of Baie des Chaleurs, Bonne Bay, western NL, based on published previous surveys)
Colodey *et al.*	1981	Off Buctouche and St-Édouard-de-Kent (northeastern NB, northern Northumberland Strait; on crabs caught in lobster traps)
Citarella	1982	Shediac Bay, NB (Veliger larvae in plankton)
D'Amours and Pilote	1982	Off La Tabatière, QC (scallop survey close to and off Lower North Shore of Gulf of St.Lawrence)
Bourget and Messier	1983	Grande Entrée harbour (Magdalen Islands, QC)
Élouard *et al.*	1983	Grande Entrée lagoon (Magdalen Islands, QC)
Lobban and Hanic	1984	North Rustico (north shore of PEI)
Wagner	1984	"Miramichi Inner Bay" (p. 4; NB)
Gilkinson	1986	(Primary records of Carter and McGregor 1979 and Hooper 1975 from NL)

Table 2. (Continued)

Authors	Year	Original or translated quotations on localities and (notes)
Citarella	1987	(Same data as in 1982 paper)
Munro and Gagnon	1989	Havre-aux-Basques lagoon and Grande Entrée harbour (Magdalen Islands, QC)
Anonymous	1997	(Site of Confederation Bridge, between Cape Tormentine, NB and Borden Point (Queens), PEI, Northumberland Strait)
Blanchard	1997	"...Escuminac Point (47°N) on the Canadian coastline.." (p. 110, based on Walne, 1956) and 59°N in Europe (fig. 2)
Bourget	1997	"Lower Estuary (north and south), Gaspé peninsula, NB, PEI" (p. 87)
Brunel *et al.*	1998	(Recorded from 5 biogeographic regions in Gulf of St. Lawrence: Baie-des-Chaleurs, Magdalen Islands, QC, around PEI, Lower North Shore of Gulf, QC and western NL)
Mitchell	1999	(Primary records of Hughes and Thomas, 1971a, 1971b, and Anonymous, 1997 and secondary ones of Dunbar *et al.* 1980 reviewed)
Collin	2001	"...from PEI, Canada, to the Bahamas" (p. 2259; based on Hoagland 1977)
Collin	2003	(Latitudinal range given in Appendix 1: between 20-30°N and >50°N)
Rosenberg	2009	Range: 48°N to 25°N; 97.2°W to 25°W; Distribution: Canada: NS, PEI, NB...

depths ranging from 7 to 82 m between Baie-des-Moutons (50°45'N, 59°01'W) and Saint-Augustin (51°13'N, 58°38'W) (D'Amours and Pilote 1982), but the exact sampling locations of *C. fornicata* were not recorded nor specimens retained. Twelve of these hauls were located in Baie des Ha! Ha! (51°00'N, 58°56'W) and nine were taken in Kecarpoui Bay (51°04'N, 58°50'W), both of which are sheltered inlets and the most likely collection sites for this species.

Data in museum and field samples

Our survey of 12 collections confirmed the general geographic pattern of *Crepidula fornicata* from our literature review (Fig. 1), with the exception of the Lower North Shore of QC for which no corresponding museum specimens could be located. In addition, museum records from along the western and eastern shores of the Gulf of St. Lawrence and southern NL coast revealed that the distribution of *C. fornicata* extends further north, northwest, and east in Canadian waters (Tables 3-4) than recognized in the recent malacological literature. With reference to the western Gulf of St. Lawrence, museum collections in the CMN still retain two of the oldest samples of *C. fornicata* collected by Bell (1859a) on the southwestern and southeastern NB shores of Baie des Chaleurs (Table 3). One of these two localities, Dalhousie NB, lies approx. 120 km inside Baie des Chaleurs from Caraquet, NB, and the associated CMN specimen (ML073451) represents the extreme northwestward limit to the distribution of *C. fornicata* in the Gulf of St. Lawrence. It is remarkable that all subsequent authors until now (Tables 1-2) have ignored the significance of this locality. Nine samples recorded in Table 3, including four collected in 2010, also extend the distribution of the species onto the northern QC shore of Baie des Chaleurs, as far as New Carlisle, in the middle of the QC shore of the bay. Only one of these nine lots is associated with a research publication (Corbeil 1953); the particular station where *C. fornicata* was dredged, however, is not specified either in the paper or on the museum label, but Corbeil's comments suggest that it may have been off Carleton, on the QC shore. In 2010, one beach walk was conducted along the shell-rich beach of Pasbébiac, east of New Carlisle; however, no *Crepidula fornicata* were found. Consequently, New Carlisle remains the furthest east along the northern QC shore of this bay where this species has been reported.

Consideration was given to mapping all null records of *Crepidula fornicata* in the western part of the Gulf of St. Lawrence, in the St. Lawrence Estuary, the Saguenay Fjord, and the North Shore of the Gulf to provide additional evidence of the distinct biogeographic boundary in this region. This would have been a formidable task, however, given the thousands of samples of benthic fauna that have been collected over the past century, spanning from the intertidal zone to the greatest depths of the Laurentian Channel (Brunel *et al.* 1998). Our reliance on positive records is thus dependent on the assumption that these coastlines and shallow water marine environments have been sufficiently explored such that if this species was present, it would have been detected.

Museum records for the eastern-most range of *Crepidula fornicata*'s distribution in NL (Table 4) also deserve special attention. Of 130 collection lots of *C. fornicata* in the CMN—by far the most significant collection of eastern Canadian *Crepidula*—two are lots collected from the southwestern coast of NL in Port au Port Bay. Likewise, the NFM currently houses 17 lots of *C. fornicata*, of which 12 are from the southern and southwestern shores of NL. The earliest record of *Crepidula fornicata* in NL is labelled "*Crepidula*" collected

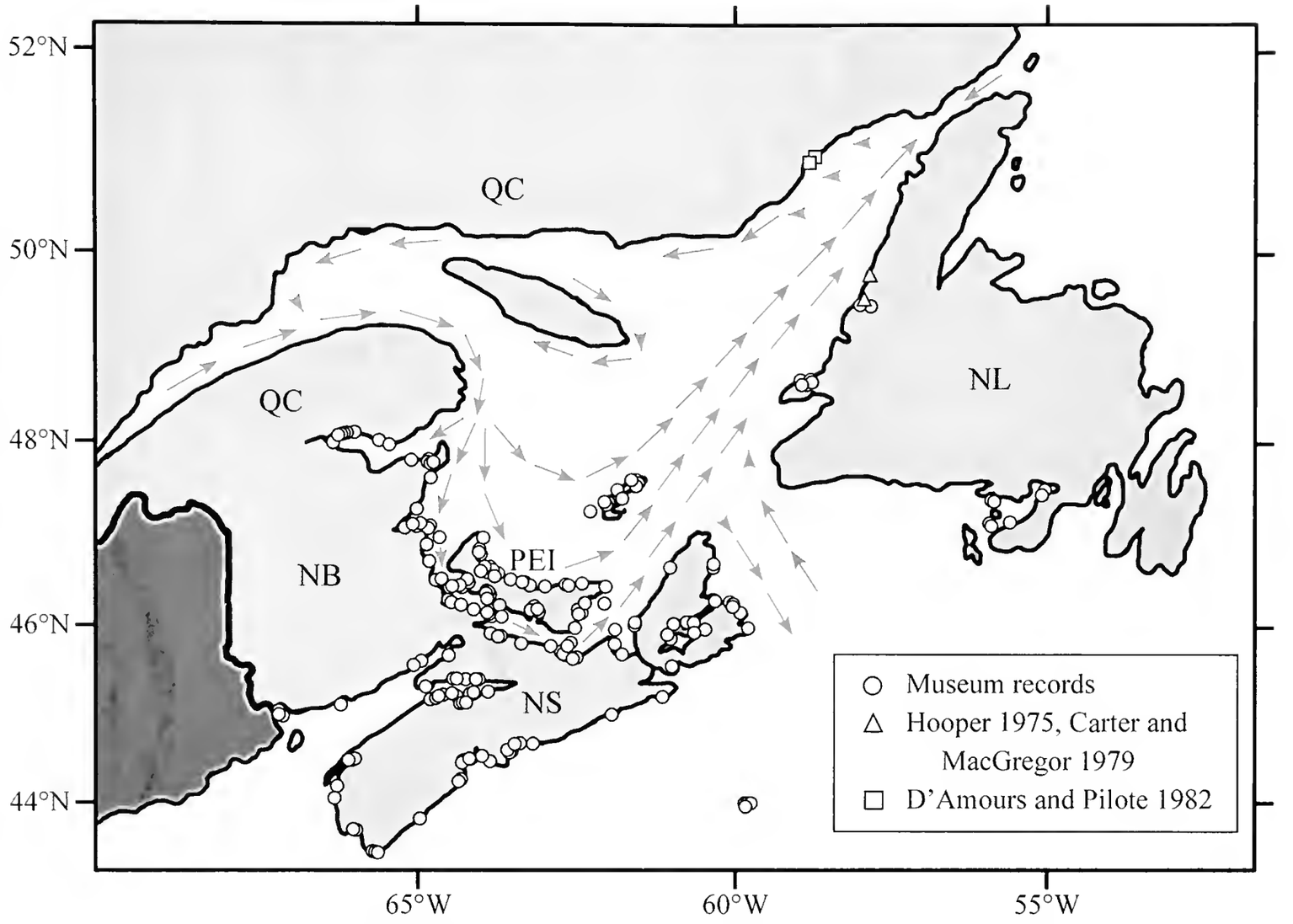

Figure 1. The distribution of *Crepidula fornicata* in eastern Canada based on collection records (1858-2010) from 12 museums in Canada and the U.S., three literature sources, and personal collections made in 2009 and 2010 (now deposited in the CMN and IQBIO). Canadian museums surveyed included the ARC, CMN, MLI, NFM, NSMNH, and ROM; U.S. museums included the AMNH, ANSP, FMNH, MCZ, NMNH, and PMNH. Where specific coordinates were associated with collection localities they were used to plot symbols. However, for many museum lots only general locality information was available for mapping. Three literature sources were also used to provide locality information for specimens of *C. fornicata* not vouchered in museum collections: Hooper 1975 (Bonne Bay and St. Paul's Inlet, NL), Carter and MacGregor 1979 (St. Paul's Inlet, NL), and D'Amours and Pilote 1982 (Lower Quebec North Shore, QC). Although specific stations associated with *Crepidula* were not noted by D'Amours and Pilote (1982), the mapped symbols represent the most likely collection localities within their broad geographic sampling of this region. Arrows represent the typical summer surface water circulation patterns within the Gulf of St. Lawrence (Koutitonsky and Bugden 1991).

at "Two Guts Pond" (48°39'N, 58°40'W), Port au Port Bay, southwest NL, by E. L. Bousfield on July 17, 1954 (CMN ML055736; Table 4). This specimen probably contributed to the change in his range description for *C. fornicata* from 1955a to 1960 to include "Chaleur Bay, NB, to southwestern Newfoundland and Nova Scotia" (Bousfield 1960). Specimens of *C. fornicata* were collected again in Port au Port Bay on June 6, 1966 by D. E. McAllister and W. Van Vliet (CMN ML091501). Beach-collected *C. fornicata* shells from Port au Port Bay are represented in the NFM collections from 1971 onwards (Table 4) and we have collected shells from this region as recently as July 2009 (shell lengths from 37.0 to 42.0 mm, $N = 3$). This 2009 sample has now been deposited at the CMN (Table 4). Although we were unable to locate any museum specimens from farther north on the western shores of NL, including Bonne Bay or St. Paul's Inlet, we collected live *C. fornicata* in Lomond, Bonne Bay, in July 2009, and specimens have also been observed and collected in other regions of Bonne Bay in 2009 (Table 4; Robert Hooper, pers. comm.). Live specimens from Bonne Bay ranged in shell length from 21.6 to 43.2 mm ($N = 4$) suggesting that these had survived at least one winter; in addition, two individuals

Table 3. Chronological data documenting selected museum samples of *Crepidula fornicata* near the northern limit of its distribution along the western shores of the Gulf of St. Lawrence, north of Pointe Sapin, NB (46°58'N, 64°50'W).

Museum[1]	Cat. No.	Date	Locality	Geographic coordinates[2]	Collector[3]	Reference
CMN	ML073451	1858[4]	Dalhousie, NB ("Bay of Chaleur", southwestern shore)	[48°04'N, 66°23'W]	RB	Bell 1859a, 1859b
CMN	ML073457	1858[4]	Caraquet, NB ("Carraquette Bay, Bay of Chaleur", southeastern shore)	[47°48'N, 64°57'W]	RB	Bell 1859a
CMN	ML091184	1950-07-28	Off Munro Island, Shippigan Bay, NB	47°45'N, 64°47'W	ELB	
CMN	ML091160	1950-08-29	Off Point aux Carr, Miramichi Bay, NB	47°05'N, 65°13'W	ELB (Stn M-140)	Bousfield 1955b (map of stations)
CMN	ML055659	1950-08-30	Off Egg Island, Miramichi Bay, NB	47°06'N, 65°03'W	ELB (Stn M-147)	Bousfield 1955b
CMN	ML091198	1950-08-30	Off Île du Vin, Miramichi Bay, NB	47°06'N, 65°05'W	ELB (Stn M-121a)	Bousfield 1955b
CMN	ML055661	1950-08-30	Off Hardwicke, Miramichi Bay, NB	47°05'N, 65°01'W	ELB (Stn M-151)	Bousfield 1955b
CMN	ML091164	1950-08-31	Off Escuminac Village, NB (southeast Miramichi Bay)	47°05'N, 64°55'W	ELB (Stn M-162)	Bousfield 1955b
NMNH	366211	1950-08	Escuminac Point, NB (southeast Miramichi Bay)	[47°04'N, 64°47'W]	ELB (Stn. M-108)	Bousfield 1955b
MLI	(Acc. 2208)	1952-10	Between Carleton, QC and Miguasha Point, QC, western Baie des Chaleurs[5]	48°05'N, 66°10'W	HEC (MAPAQ Stn D42)	Corbeil 1953 (basis for Brunel 1970a)
CMN	ML031227	1960-08-04	North channel of lagoon, Tracadie Bay, NB	47°33'N, 64°53'W	ELB (Stn B-13)	
CMN	ML038997	1960-08-05	St. Simon Inlet, Shippigan Bay, NB	47°45'00"N, 64°49'00"W	ELB (Stn B-17)	
CMN	ML073442	1961-07-13	"MRC de Bonaventure" (northwestern Quebec shore of Baie des Chaleurs)	[48°05'N, ~66°W]	AET	
CMN	ML067226	1966-07-20	St. Simon Inlet, Shippigan Bay, NB	47°46'00"N, 64°46'00"W	MLHT	
CMN	ML067153	1966-07-21	West side of Brule Point, Pointe-Brûlé, Shippigan Bay, NB	[47°45'36"N, 64°44'49"W]	MLHT	
CMN	ML067270	1966-07-21	Neguac Plots, NB (north of Miramichi Bay)	47°15'N, 65°05'W	MLHT	
MLI	(Acc. 410)	1982-09-01	New Carlisle, QC (north shore of Baie des Chaleurs)	48°00'15"N, 65°23'00"W	DDA	
CMN	ML092899	1990-08-11	Beach at Carleton, QC (northwestern shore of Baie des Chaleurs)	[48°06'N, 66°08'W]	MC	
FMNH	282288	2000	Escuminac Point, NB (southeast Miramichi Bay)	47°06'N, 64°49'W	DV (DV-282288)	Collin 2001
IQBIO[6]	41-	2000-08-01	Subtidal sand with *Zostera* at Carleton, QC	48°06'44"N, 66°08'48"W	PB	
IQBIO	41-	2010-07-27	Subtidal sand with *Zostera* at Carleton, QC	48°05'44"N, 66°08'48"W	PB	
IQBIO	41-	2010-07-28	Beach east of Carleton, QC	48°06'00"N, 66°04'40"W	PB	
IQBIO	41-	2010-07-29	Beach west of New Richmond, QC	48°10'22"N, 65°53'02"W	PB	
IQBIO	41-	2010-07-29	Beach south of Bonaventure, QC	48°01'28"N, 65°28'23"W	PB	

[1]Museum abbreviations: CMN, Canadian Museum of Nature; FMNH, Field Museum of Natural History; IQBIO, Institute Québécois de la Biodiversité; MLI, Maurice Lamontagne Institute; NMNH, National Museum of Natural History. [2]Geographical coordinates enclosed in brackets were approximated based on site locality information. [3]Author abbreviations: RB, R. Bell; ELB, E. L. Bousfield; HEC, H.-É. Corbeil; AET, A. E. Tener; MLHT, M. L. H. Thomas; DDA, D. D'Amours; MC, M. Castonguay; PB, P. Brunel; DV, D. Véliz. [4]Listed as 1866 in museum records; however, Bell, who sampled molluscs in this bay in 1858, is not known to us to have been there in 1866. The number of specimens agrees with Bell (1859a); [5]Locality based on notes in Corbeil (1953) pertaining to 14 species of molluscs collected: "a small number of specimens were taken", and "apart from a few specimens dredged in the deeper muddy areas, molluscs are encountered mostly near shore, and in five sections off Carleton." (p. 63); [6]Provisional storage of a private collection in MLI started in October, 2008; final catalogue numbers have yet to be assigned.

Table 4. Chronological data documenting selected museum samples of *Crepidula fornicata* near the northeastern limit of its native distribution in western and southern Newfoundland (NL).

Museum[1]	Cat. No.	Date	State	Locality	Geographic coordinates[2]	Collector[3]	Reference
CMN	ML055736	1954-07-17	Wet	Two Guts Pond, Port au Port Bay, W NL	48°39'N, 58°40'W	ELB (StnW-12)	Bousfield 1960
CMN	ML091501	1966-06-09	Dry	Port au Port Bay, (park at S end of W. Bay), W NL	[48°33'31"N, 58°54'26"W]	DEM, WVV	
NFM	MO-1974	1971	Dry	1.5 miles NE of Harbour Mille, NE Fortune Bay, S NL	[47°35'18"N, 54°51'20"W]	JEM, RGN	
NFM	MO-1980	1971	Dry	Grand Beach, Fortune Bay, S NL	[47°08'33"N, 55°31'20"W]	RGN	
NFM	MO-1981	1971	Dry	Piccadilly, Port au Port Bay, W NL	[48°33'28"N, 58°54'16"W]	RGN	
NFM	MO-1975	1980	Dry	Southern Fortune Bay, S NL	[47°10'N, 55°46'W]	TA	
NFM	MO-1976	1980	Dry	Southern Fortune Bay, S NL	[47°10'N, 55°46'W]	DM	
NFM	MO-1982	1982	Dry	Harbour Breton, Fortune Bay, S NL	[47°28'56"N, 55°47'48"W]	RGN	
NFM	MO-1977	1983	Dry	Boswarlos, Port au Port Bay, W NL	[48°34'10"N, 58°48'55"W]	RGN	
NFM	MO-501	1984	Dry	Black Point, Port au Port Bay, W NL	[48°36'16"N, 58°41'10"W]	JEM	
NFM	MO-502	1984	Dry	West Bay Beach, Port au Port Bay, W NL	[48°35'54"N, 58°56'16"W]	JEM, BW	
NFM	MO-1768	1988	Dry[4]	Piccadilly, Port au Port Bay, W NL	[48°33'28"N, 58°54'16"W]	JEM	
NFM	MO-1978	1995	Dry	Little Bay East, NE Fortune Bay, S NL	[47°33'29"N, 54°50'54"W]	JEM	
NFM	MO-1979	1998	Dry	Harbour Breton, Fortune Bay, S NL	[47°28'56"N, 55°47'48"W]	RGN	
CMN	ML094274	2009-05	Wet	Norris Point, Bonne Bay, Gros Morne National Park, W NL	49°31'03" N, 57°52'33"W	RH	Present paper
CMN	ML094275	2009-07-20	Dry	Port au Port Bay, (park at S end of E. Bay), W NL	48°33'32" N, 58°43'47"W	TAR, JMA, and MJM	Present paper
CMN	ML094276	2009-07-21	Wet	Lomond, Gros Morne National Park, W NL	49°27'44" N, 57°45'38"W	TAR, JMA, and MJM	Present paper

[1]Museum abbreviations: CMN, Canadian Museum of Nature; NFM, The Rooms Provincial Museum. [2]Geographical coordinates enclosed in brackets were approximated based on site locality information. [3]Collector abbreviations: BW, B. Withycombe; DM, D. Manuel; DEM, D. E. McAllister; ELB, E. L. Bousfield; JMA, J. M. Aker; JEM, J. E. Maunder; MJM, M. J. MacInnis; RGN, R. G. Noseworthy; RH, R. Hooper; TA, T. Ansty; TAR, T. A. Rawlings; WVV, W. Van Vliet; [4]Subfossil: based on a visual evaluation by J. Maunder, NFM.

forming a stack were both found to be brooding eggs. Two voucher shell lots from this region, including material preserved in alcohol, are now deposited at the CMN (Table 4).

The NFM also retains 7 lots of *C. fornicata* from along the southern shores of NL. These localities are not currently reflected in any primary research publication. The earliest records date to 1971 when two collections of *C. fornicata* were made in southeastern and northeastern Fortune Bay, on the southern coast of NL (Fig. 1; Table 4). Subsequent collections have been made in other areas of Fortune Bay, the most recent in 1998 (Table 4). In October 2007, *C. fornicata* was also observed on mussel shells in Pool's Cove, Fortune Bay, by a field researcher at Memorial University's Ocean Sciences Centre (Philip Sargent, pers. comm.).

DISCUSSION

Museum collections have long been employed to document changes in the geographic distribution and morphology of marine gastropods through time (*e.g.*, Vermeij 1982, Seeley 1986, Carlton *et al.* 1991, Fisher *et al.* 2009). Here, we have used records from 12 museums, supplemented with a literature review and new collections, to clarify the present distribution of a well-known shallow water marine gastropod, *Crepidula fornicata*, in the northwestern Atlantic. Our results extend the northern distribution of *C. fornicata* in eastern Canada potentially as far north as 51°N latitude and as far east as 55°W. Although the northern distribution of *C. fornicata* is still substantially lower than the 59°09'N achieved by introduced *C. fornicata* in Oslo Fjord, Norway (Blanchard 1997), northeastern Atlantic water temperatures are typically warmer at a given latitude than in the northwestern Atlantic. As for many other intertidal and shallow marine organisms, water temperature likely plays an important role in limiting the northerly distribution of *Crepidula* (Hutchins 1947, Bousfield and Thomas 1975, Southward *et al.* 1995, Therriault and Herborg 2008). Thieltges *et al.* (2004) have associated cold temperatures with heavy winter mortality and low abundance of *C. fornicata* in the northern Wadden Sea of Germany (54°49'–55°09'N), where long-term average water temperatures range from 2.7 °C in February to 18.1 °C in August. Abundance of *Crepidula* barely reaches 100/m^2 at this latitude compared to >1000/m^2 in more southern localities in Europe, and overwinter mortalities can reach 97% in some mussel beds (Thieltges *et al.* 2004). Mortality is assumed to be the result of ice scour or direct exposure to low temperatures, particularly during aerial emersion in intertidal or shallow subtidal habitats.

The distribution of *Crepidula fornicata* in the northwestern Atlantic likely reflects: (1) long-term post-glacial changes in the Canadian Atlantic, (2) seasonal physical conditions within shallow water marine habitats, (3) physiological tolerances to water temperature, salinity, and dissolved oxygen, and (4) current patterns associated with larval delivery. Environmental niche modeling (Therriault and Herborg 2008) can help to link known localities of a species to critical environmental parameters in order to predict its potential distribution. The output of such models often predicts the non-contiguous distribution of a species (Therriault and Herborg 2008), where its presence is associated with patches of favourable physical conditions (Bousfield and Laubitz 1972). Such modeling appears most effective when it incorporates seasonal variation in temperature and salinity rather than summer or winter values alone. In the shallow water suspension-feeding ascidian *Ciona intestinalis*, of 10 environmental variables used to predict the distribution, three variables (October – December water temperature, October – December salinity, and January-March water temperature) were the highest contributors to model accuracy (Therriault and Herborg 2008).

While niche modelling has yet to be done for *Crepidula fornicata*, the distribution of this species in eastern Canada does correspond with surface water temperatures. Within the southwestern Gulf of St. Lawrence, for instance, the northern distribution of *C. fornicata* ends at the middle of the north shore of Baie des Chaleurs near New Carlisle, QC (Fig. 1). The abrupt end to its distribution is associated with the presence of significantly colder waters in the northeastern, open portion of Baie des Chaleurs (Bousfield and Thomas 1975). A branch of the cold Gaspé Current moves westward along the northeastern QC shore of this bay (Tremblay 1944, Brunel 1959) and deviates into a cyclonic gyre in the middle of the bay (Legendre and Watt 1970). The abundance of collection records of *C. fornicata* in the warm southern Gulf, Bras d'Or lakes, and Minas Basin, corresponds with the presence of warm (>18 °C) summer surface waters (Bousfield and Thomas 1975). This pattern is not absolute, however. *Crepidula fornicata* occurs in near shore environments with colder summer water temperatures, including the Magdalen Islands, Cape Breton Highlands and Sydney Bight (15-18 °C), as well as the outer coast of NS, including the Bay of Fundy (12-15 °C) (Bousfield and Thomas 1975). In these localities, *C. fornicata* is apparently confined to sheltered inlets and bays where temperatures are warmer (Bousfield and Laubitz 1972). In NL, *Crepidula* is now known to be associated with the warmer summer waters of Port au Port Bay (15-18 °C) on the southwestern coast, and within bays and inlets of Fortune Bay on the southern coastline, where the summer temperature range is typically 12-15 °C (Bousfield and Thomas 1975). This summer temperature range also corresponds to localities of *Crepidula* in Bonne Bay and St. Paul's Inlet on the west coast. In Bonne Bay, surface water temperatures typically range from -1 to 16-18 °C (Hooper 1975), corresponding well with

our collection of *C. fornicata* in Lomond, July 21, 2009, where water temperature was 14.8 °C (0.5 m depth). Likewise, Carter and MacGregor (1979) reported typical summer surface water temperatures of ≤15 °C in St. Paul's Inlet during their 1977-78 sampling.

The discovery of *Crepidula fornicata* along the Lower North Shore of QC (D'Amours and Pilote 1982; "BCN" in Brunel *et al.* 1998) is the most surprising distributional record of *C. fornicata* based upon water temperature. This region is generally the coldest area of the Gulf of St. Lawrence, receiving icebergs from Labrador through the Strait of Belle Isle, with summer surface temperatures typically below 12 °C (Bousfield and Thomas 1975, Brunel *et al.* 1998). Nevertheless, the Lower North Shore also has numerous sheltered bays and inlets. Growth rates of giant scallop (*Placopecten magellanicus* (Gmelin, 1791)) in this region can also be very high, suggesting that warm plankton-rich microclimates, suitable for other suspension-feeding organisms such as *Crepidula*, are present locally (Pilote, pers. comm.).

Has *Crepidula fornicata* expanded its distribution into NL and the northeastern Gulf of St. Lawrence within the past 50 – 60 years, or are these new records simply a reflection of wider geographic sampling? The sub-fossil *C. fornicata* shells collected in 1988 from Picadilly, Port au Port Bay (J. Maunder, pers. comm., Table 4), suggest that this species has existed along the NL coastline in the past. These undated fossils are not necessarily reflective of the long-term presence of this species in Port au Port Bay, however, and may indicate their transient presence during past periods of warmer ocean temperatures (Bousfield and Thomas 1975). The first recent evidence of *C. fornicata* in NL, beyond Baie des Chaleurs, QC, was one lot collected by Bousfield from Port au Port Bay in 1954, followed by collections by McAllister and Van Vliet in 1966, and observations by Mercer in 1968. In part, the prior absence of records in NL may reflect the dearth of interest in non-commercial benthic marine molluscs of Newfoundland and Labrador in the 1900s (Gilkinson 1986; but see Dall 1926). The rugged coastline of NL, particularly the southern coast between Port aux Basques and Fortune Bay, has also limited surveys, and is the most poorly studied coastal region of NL (Gilkinson 1986). An additional factor may be the low population density of this species in NL. In areas that we have sampled, this species is simply not as abundant as in similar habitats at lower latitudes and does not appear to contribute to beach shell debris to the same extent as it does further south. Hence, this species is much less conspicuous within the northern extremes of its range in the northwestern Atlantic.

The planktotrophic development of this species and its long larval life of several weeks (Pechenik 1990) nevertheless provide opportunity for *Crepidula fornicata* to exploit new environments when conditions are suitable. The rapid spread in its introduced range in Europe is due in part to dispersal of larval stages (Blanchard 1997). The direction of surface currents, irrespective of temperature, may either stop or enhance larval dispersal. For instance, the branch of the cold Gaspé Current which deviates westward into the northeastern half of Baie des Chaleurs due to the Coriolis effect (Tremblay 1944, Brunel 1959) may well shift larvae into the central cyclonic gyre (Legendre and Watt 1970) and carry them back toward the southeastern or southwestern shores of the bay. In contrast, the August circulation off the western NL shore is clearly directed toward the Lower North Shore of the Gulf of St. Lawrence (El-Sabh 1976, Koutitonsky and Bugden 1991) in the most propitious season. This pattern of currents, coupled with the favourable inlet habitats along this coastline, could thus explain the surprising occurrence of *C. fornicata* in this otherwise cold environment.

Crepidula fornicata has also had a long history of human-mediated range expansion through the transport of oysters and mussels for aquaculture as well as via the fouling of boat hulls (Carlton 1992, Blanchard 1997, Collin *et al.* 2006). Aquaculture operations, such as introduction of oysters into habitats of the QC shore of Baie des Chaleurs (Corbeil 1948, 1949) and in Port au Port from PEI (Mercer 1970), could certainly have introduced *Crepidula* to the northern shores of Baie des Chaleurs and to NL. Unsuccessful introduction of oysters in the Port-Daniel lagoon, northeastward from New Carlisle, could nevertheless have allowed larvae to drift back westward, and may have helped establish the species in New Carlisle. The first observation of *Crepidula* in Two Guts Pond, NL, however, precedes any known oyster introduction to western NL. Other oyster introductions are also known to have occurred in other regions of NL (Mercer 1970) although details of these are lacking. Both natural and human-mediated transport could thus be responsible for the spread of this species in NL and QC.

Interestingly, *Crepidula plana*, the only other common species of *Crepidula* in the Gulf of St. Lawrence, does not appear to be present in NL or the northern Gulf. This species can be found sympatrically with *C. fornicata* in some habitats in NS (T. Rawlings and J. Aker, pers. obs.), but appears to have a more restricted geographic distribution within the Gulf (Brunel *et al.* 1998). Differences in the spread (and invasion potential) of these two species have also been noted elsewhere. At least one specimen of *C. plana* was introduced into Ireland in 1865 (Blanchard 1997), but this species never became established. In contrast, Europe continues to live with the consequences of the introduction of *C. fornicata* in the late 1800s.

In summary, clarification of a species' geographic range, as attempted here for *Crepidula fornicata*, remains an important task for establishing temporal benchmarks against which to assess the effect of climate change on the marine

environment. To continue this process in eastern Canada, further exploration of the long-neglected rugged coastlines of the northern shores of the Gulf of St. Lawrence, and around NL, is clearly needed, with emphasis on the collection and preservation of voucher specimens of near-shore species, and the careful documentation of collection localities and associated physical variables.

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Brooding patterns in three freshwater mussels of the genus *Elliptio* in the Broad River in South Carolina

Jennifer E. Price[1]* and Chris B. Eads[2]

[1] Fisheries Research Program, South Carolina Department of Natural Resources, 1921 VanBoklen Road, Eastover, South Carolina 29044, U.S.A. and Belle W. Baruch Institute for Marine and Coastal Research, University of South Carolina, Columbia, South Carolina 29208, U.S.A.

[2] College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, North Carolina 27606, U.S.A.

Correspondence, J. E. Price: jenniferjohnsonprice@gmail.com

Abstract: We investigated brooding patterns and timing of reproduction in three species of freshwater mussels, *Elliptio complanata* (Lightfoot, 1786), *Elliptio roanokensis* (Lea, 1839), and *Elliptio angustata* (Lea, 1831) in the lower Broad River near Columbia, South Carolina. Through repeatedly marking and recapturing individuals from late March through late June 2008, we determined that *E. complanata* and *E. roanokensis* can complete at least two broods during this season, and that *E. angustata* can complete at least three broods with very little time between broods. The lengths of brooding periods were difficult to estimate and subject to a wide margin of error. However, the brooding period was estimated to be 20 to 36 days in most broods of *E. angustata*, and could be as short as 18 days in *E. roanokensis* and 25 days in *E. complanata*. Mark and recapture of gravid mussels provided a much more detailed picture of brooding strategies than the collection and preservation of different individuals throughout the year.

Key words: *Elliptio complanata*, *Elliptio roanokensis*, *Elliptio angustata*, consecutive reproduction, broods

Freshwater mussels exhibit a wide variety of brooding patterns. Mussels are classified as short-term brooders (tachytictic), in which the female contains developing glochidia for approximately one month, and long-term brooders (bradytictic), in which broods typically last between 8 and 10 months (Ortmann 1912). Detailed literature reviews (Heard 1998, Watters and O'Dee 2000) indicated that although these two classifications have been in use for a long time, a wider variation in reproductive strategies exists. The length of the brooding period may also vary depending upon temperature cues (Watters and O'Dee 2000). In addition to variation in brooding patterns among species, there may be variation among individuals, and among populations in different geographic areas (Heard 1998). Mussels may also brood only once per year (sequential reproduction) or produce more than one brood each year with a minimal time lapse between broods (consecutive reproduction) (Heard 1998).

Brooding patterns, such as the number of broods per year, the season during which mussels brood, and the tendency of mussels to pause or skip a year of reproduction may differ between populations or between years based upon geographic and environmental factors (Hochwald 2001). Understanding responses to these variables may assist conservation efforts aimed at relieving environmental stresses and increasing reproductive output (Hochwald 2001). Estimating the duration of the brooding season may assist conservationists in regulating activities to minimize disturbance to mussel populations during a particularly sensitive time in their life cycle. Knowledge of the frequency and timing of brooding within a population will also assist researchers in studying evolutionary aspects of a population by estimating fecundity and response to environmental or biological changes. The timing of broods and glochidial release may be especially critical for species that use migratory hosts, or hosts whose activity or feeding behavior varies seasonally. Understanding fecundity, brood production, and brood maturation is also beneficial for propagation programs for rare species.

There are a few documented examples of freshwater mussels that complete 2 broods per year with or without a lengthy rest period in between (Heard 1975, Smith 1978, Parker *et al.* 1984, Jones *et al.* 1986, Gordon and Smith 1990, Watters and O'Dee 2000) although cases in North America are fairly rare. A literature review (Hochwald 2001) indicates that most European species of *Unio* Philipson, 1788 can undergo multiple reproductive cycles within one season.

* Present address: Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

We tracked the reproductive status of individual mussels over time and examined the tendency to brood multiple times per season in 3 species in the genus *Elliptio* (Unionidae) Rafinesque, 1819: *Elliptio complanata* (Lightfoot, 1786), *Elliptio roanokensis* (Lea, 1839), and *Elliptio angustata* (Lea, 1831). All of these species are ectobranchous (brooding glochidia in the outer gills only). Hermaphroditism has been known to occur at a low frequency in some populations of *E. complanata* (Matteson 1948, Heard 1979, Downing *et al.* 1989). It is not known whether *E. roanokensis* is monoecious or dioecious, but Van der Schalie (1970) noted that *Elliptio producta* (Conrad, 1836) and *Elliptio dilatata* (Rafinesque 1820), members of the same species complex as *E. angustata*, were occasionally hermaphroditic, but allocated most of their reproductive tissue to one sex. As *Elliptio* species are not sexually dimorphic, it was not possible to determine whether individuals in this study were male, female, or hermaphrodites without removing gonadal tissue.

MATERIALS AND METHODS

Study site

The study site in the lower Broad River in Columbia, South Carolina contained a diverse community of mussels. The site was at the upstream end of a side channel among an island complex that forms the confluence of the Broad and Saluda Rivers, in Richland County (34.00421°N, 81.05748°W). The substrate was composed of sand, gravel, and large boulders. The site was near the fall line and below several small riffles producing a fast current. Previous survey work the year before showed that the area had a diverse and moderately abundant mussel community, containing *Lampsilis radiata* (Gmelin, 1791), *Ligumia nasuta* (Say, 1817), *Strophitus undulatus* (Say, 1817), *Villosa delumbis* (Conrad, 1834), *Uniomerus carolinianus* (Bosc, 1801), *Elliptio complanata*, *Elliptio congaraea* (Lea, 1831), *Elliptio roanokensis*, and *Elliptio angustata*. Only one non-gravid individual of *S. undulatus* was found at the study site. *Uniomerus carolinianus* and *E. congaraea* were also uncommon, and only one and 3 gravid individuals of each species respectively were found at the study site. As *L. radiata*, *L. nasuta*, and *V. delumbis* are bradytictic, they did not release multiple broods within a season, and their reproductive patterns will be discussed elsewhere.

Field procedures

On 8 days in the summer of 2008, we collected mussels by snorkeling or a combination of scuba diving and snorkeling, depending upon water depth. We opened the mussels by partially prying apart the valves along the ventral margin, and classified them as gravid or not gravid. Gravid was defined as having visible swelling with tubes of glochidia on the outer gills. On each survey, all non-gravid mussels were etched with a single digit number using a Dremel® tool to indicate the survey date. This allowed us to document the reproductive history of non-gravid mussels should they later be found gravid, while still being able to quickly return them to the river to minimize desiccation and thermal stress. The first time a mussel was found to be gravid, a numbered flexible plastic 4 mm × 8 mm tag (Hallprint Pty Ltd., Victor Harbor, South Australia) was super-glued to the shell. The tag could be used to track the reproductive status of the mussel through time. After all mussels were examined and marked accordingly, they were immediately returned to the river in a small area (roughly 15 m^2) that provided suitable habitat but allowed the mussels to be easily relocated. On each successive date, we thoroughly searched this area to recapture mussels in addition to searching a broader area to locate new individuals.

Sampling dates were 27 March, 17 April, 2 May, 14 May, 30 May, 4 June, 17-18 June, and 25 June 2008. On 17 June 2008 a thunderstorm interrupted field work, and the examination and marking of mussels was completed on the following day. Mussels not yet examined were kept in the river in collection bags with wide enough mesh to allow adequate water flow. The start date of sampling was limited by cold water temperatures and high flows, which caused poor visibility and safety concerns for field work. High flows during early April also forced a delay in the second sampling date and a deviation from the desired schedule of sampling at least once every 2 weeks. A decision to conclude the study was made after relatively few gravid individuals were observed on 25 June 2008, indicating that the brooding season was probably nearing its end.

Between 80 and 110 mussels were examined on each date. Recapturing individuals was critical to documenting brooding patterns, and an attempt was made to retrieve as many previously marked individuals as possible. However, retrieving every individual was not always possible, and we tried to achieve a balance between concentrating effort on relocating previously marked individuals and searching for additional individuals of less common species for which reproductive data were lacking.

Multiple broods were determined in individuals found on multiple sampling events and observed to alternate between gravid and not gravid. Because not all mussels were recaptured each time, we present data on the maximum length of the brooding period based only on mussels that were observed on at least three consecutive sampling events and were observed when not gravid, when subsequently gravid, and after brood release.

A Chi-square test compared the tendency of each species to have a second brood. Statistical analysis was conducted in SAS for Windows, version 9.1, SAS Institute, Cary, North Carolina.

Water temperatures

Because temperature can be an important cue in influencing the reproduction of freshwater mussels (Heinricher and Layzer 1999, Watters and O'Dee 2000, Hastie and Young 2003) we report water temperatures for the nearest USGS guage (USGS 2010) upstream of the study site (Fig. 1). Temperatures downstream are not considered to be similar to those at the study site because of the confluence of another river with a large dam and unusually cold water temperatures. The monitoring station or guage, USGS site 02160991, is located 41.5 km upstream of the study site, at 34.258333°N, 81.330556°W.

RESULTS

Timing of broods

Elliptio complanata was first gravid beginning on 2 May 2008, and gravid individuals were found continuously through the end of the study on 25 June. Percentages of gravid individuals ranged from 46% on 2 May to 4% on 25 June (Fig. 2A). *Elliptio roanokensis* was gravid on all study dates, and rates of gravid individuals ranged from 35% on 27 March to 3% on both 30 May and 25 June (Fig. 2B). *Elliptio angustata* was also gravid on all study dates, and the frequency of gravid individuals ranged from 56% on 4 June to 8% on 25 June (Fig. 2C).

Number of broods

We observed 14 gravid *Elliptio complanata* out of 36 individuals captured and marked throughout the season. Through multiple recaptures, we confirmed the occurrence of a second brood in 2 of these individuals. If an individual was gravid twice, we needed to recapture it at least twice following the release of the first brood to document the second brood, and even so, the second brood may or may not have been observed, depending upon the dates of capture versus the dates of the brood. Of the 12 individuals in which we observed one brood, only 4 were recaptured at least twice following the release of the brood.

We observed 19 gravid *Elliptio roanokensis* out of 60 individuals captured and marked. Through multiple recaptures, we confirmed a second brood in 3 of these individuals. Individuals of *E. roanokensis* were more likely to be recaptured than either *Elliptio complanata* or *Elliptio angustata*, probably due to the larger body size and ease of locating individuals. Of the 16 individuals in which only one brood was observed, only 3 were not recaptured, 2 were recaptured only once, and the remaining 11 individuals were recaptured between 4 and 6 times each. Although it is possible that a very late or very early season brood was missed in these individuals, it is likely that many of the individuals recaptured 4 to 6 times following the release of the first documented brood actually brooded only once.

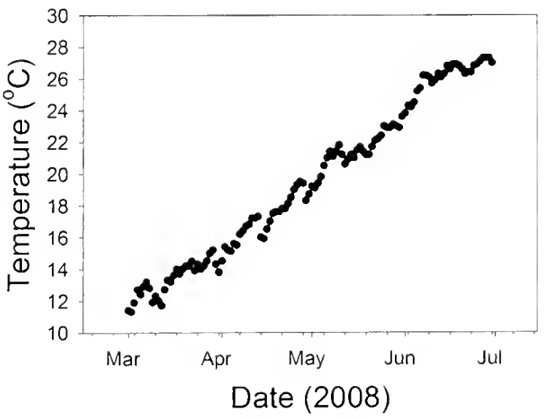

Figure 1. Water temperatures at USGS guage 02160991, Broad River, near Jenkinsville, South Carolina.

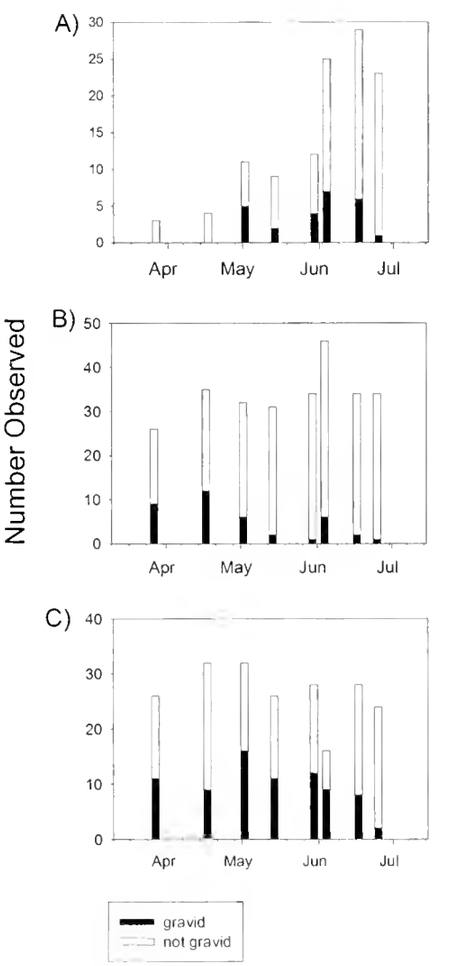

Figure 2. Reproductive status of mussels observed by date: A, *Elliptio complanata*; B, *Elliptio roanokensis*; and C, *Elliptio angustata*. Major tick marks indicate the first day of the month labeled in 2008.

We observed 32 gravid *Elliptio angustata* out of 77 individuals captured and marked during the study. Multiple broods were documented in 8 individuals—4 with 2 broods and 4 with 3 broods. Twenty-four individuals were documented with only one brood. Of these, 15 were not recaptured alive after releasing the first brood, including 3 that were later found dead, as empty relic shells. Three were recaptured only once, 5 were recaptured twice, and one was recaptured 3 times. Of individuals that were observed gravid at least once, all but one of them was captured at least 5 times, and all individuals captured at least 7 times were documented with at least 2 broods, indicating that multiple broods may be more common than a single brood per season. For individuals captured a minimum of 5 times, *E. angustata* was significantly more likely (8 out of 9) than *Elliptio roanokensis* (3 out of 14) to be observed carrying a second brood (Chi-square test, χ^2= 9.99, df = 1, P = 0.0016). Only 3 *Elliptio complanata* were captured at least 5 times, which is not enough for inclusion in this analysis.

Length of time between broods

All 3 species were able to begin the second brood fairly quickly after release of the first brood. Of the 2 *Elliptio complanata* observed with 2 broods, the range of possible times between broods were 1 to 20 days and 1 to 29 days. Of the 3 *Elliptio roanokensis* observed with 2 broods, the range of possible times between broods were 29 to 47 days, 1 to 27 days, and 1 to 35 days.

Of the 8 *Elliptio angustata* that were observed with 2 or more broods, the minimum possible time between the first and second brood (since the status of the mussel between capture times was unknown) ranged from 1 to 29 days (mean = 10.25 ± 10.72 *SD*). The maximum possible time between these broods ranged from 26 to 61 days (mean = 39.75 ± 11.17). For the 4 individuals observed carrying a third brood, the time between broods appeared to be even shorter. All 4 individuals observed carrying a third brood were recaptured only once between the second and third brood, making the minimum possible time between broods always equal to one day. The maximum time between broods was either 17 days (one individual) or 20 days (3 individuals). The apparently shorter interval in individuals observed carrying 3 as opposed to 2 broods may be an artifact of sampling rather than an actual tendency of the second and third broods to be closer in time than the first and second. Individuals that experience the shortest time lapse of any individuals in the population between broods are more likely to be observed with three broods.

Length of brooding period

We calculated a minimum possible brooding period for 9 broods in *Elliptio complanata* that were collected at least twice in the gravid state without being collected in the non-gravid state in between. Minimum brooding periods ranged from 6 to 16 days (mean = 11.9, *SD* = 4.0, median = 14). A maximum possible brood length could be estimated only for 3 individuals, because many were not recaptured after the last time they were found to be gravid. The maximum brooding periods were 25, 33, and 42 days.

We determined a minimum brooding period for 9 broods in *Elliptio roanokensis*. Seven of these were individuals that were gravid for a minimum of 17 days, and one was gravid for at least 15 days. The remaining individual was gravid on surveys 55 days apart without being recaptured in between, so this observation was considered an outlier, possibly representing two separate broods. The maximum brood length could only be estimated for 5 individuals since the greatest number of individuals that were gravid was the first day of the study (Fig. 2) and it was not known how long these were already gravid. Maximum brood estimates were 18, 24, 26, 34, and 42 days.

A minimum possible brooding period was determined for 17 *Elliptio angustata*. In one individual, the brood appeared to be in the very early stage during one of the later gravid recaptures. Since this probably indicates that 2 broods occurred during the time these mussels were collected and classified as gravid, this individual was excluded from estimates of brood duration. Minimum potential brood lengths ranged from 6 to 48 days (mean = 23.5 ±11.7). The 48 day minimum brood appeared to be an outlier, and the next longest estimate of a minimum brood was 36 days. We were able to calculate maximum brood lengths for 10 individuals that were captured as not gravid, later gravid, and non gravid even later, so that the individual was determined to be gravid no longer than the intervening days between non-gravid captures. Maximum potential brood duration ranged from 17 to 62 days (mean = 37.8 ± 17.4 days). Though the possibility exists that some of the broods that appeared to be the shortest were prematurely aborted broods, and some that appeared to be particularly long could have actually been 2 broods, the estimate that broods generally ranged in length from 20 to 36 days would allow 16 out of 17 estimates of minimum brood length and 9 out of 10 estimates of maximum brood length to fall within this range.

Sizes of brooding individuals

Elliptio complanata ranged from 39 to 95 mm in length. Gravid individuals ranged from 61 to 89 mm. *Elliptio roanokensis* ranged from 48 to 141 mm in length, and gravid individuals were from 65 to 130 mm in length. The 65 mm individual was unusual and may have been an error or a misidentified *E. complanata* because the next smallest gravid *E. roanokensis* was 88 mm in length. *Elliptio complanata* and *E. roanokensis* are difficult to distinguish when small. *Elliptio*

angustata ranged from 44 to 104 mm in length. Gravid individuals ranged from 53 to 104 mm in length.

Water temperatures

Average daily water temperatures were 14.5 °C at the start of the study (Fig. 1) when numerous *Elliptio angustata* and *Elliptio roanokensis* were already gravid. Average daily water temperature was 19.1 °C on 2 May 2008 when *Elliptio complanata* was first gravid and 17.0 °C on 17 April 2008, the last day that no gravid individuals were found (Fig. 1).

DISCUSSION

Elliptio complanata and *Elliptio roanokensis* were able to complete at least 2 broods in a season, and *Elliptio angustata* was able to complete at least 3 broods. Three is a minimum number, and we may not have been able to detect additional broods, even if they did occur. For example, with only 8 sampling dates, the maximum number of broods that could be detected is 4, and if 4 did occur during the sampling period, they would be detectable only in individuals recaptured at least 7 times on dates that coincided with alternating gravid and non-gravid periods. Because many gravid *E. roanokensis* and *E. angustata* were collected on the first sampling date, it is unknown how early the brooding season started, and additional broods may have occurred prior to sampling. We appear to have documented the beginning of the brooding season for *E. complanata* because no gravid individuals were found prior to 2 May 2008. Very few individuals were found prior to 2 May (only 3 on 27 March and 4 on 17 April), and it could be that none were found gravid by chance. However, *E. complanata* may migrate below the substrate surface in winter, and emerge at the surface during peak periods of reproductive activity in the spring (Amyot and Downing 1991, Balfour and Smock 1995). Therefore, it is likely that individuals that could not be located occurred below the surface and were not yet reproducing. The low numbers of *E. complanata* early in the study resulted in low recapture rates and little information on the number of broods for this species.

Many studies on brooding patterns in freshwater mussels (reviewed in Bauer and Wächtler 2001 and in Heard 1998) involved sacrificing individuals at periodic intervals, tracking patterns on the population rather than individual level. This can make it difficult to determine the length of the brooding period or whether multiple broods occur. One of the few studies that did track individuals involved the European species *Unio crassus* Philipsson, 1788 in which multiple broods were observed in individuals by checking their reproductive status weekly (Hochwald 2001). More frequent examination of marked mussels would have given us a more accurate picture of brooding patterns but also might have disrupted reproductive behavior and increased the possibility of injury. However, our methodology clearly documented that some individuals undergo multiple broods in one season and illustrated the benefits of using mark-recapture techniques in reproductive studies.

There appeared to be variation in the number of broods produced by an individual in *Elliptio roanokensis*. While there is a possibility that some individuals observed with one brood actually produced more that were not observed, *E. roanokensis* individuals were frequently recaptured many times following the release of a brood and only a few of those were observed with a second brood. The larger body size of *E. roanokensis* may have again made it easier to locate than smaller species. *Elliptio angustata* may have been more consistent in the number of broods produced since most individuals recaptured at least five times had at least two broods. Not enough data were available to draw conclusions about the prevalence of multiple broods in *Elliptio complanata*. Although little information on variation in brooding patterns among individuals in a single population is available, Hochwald (2001) found that the number of broods per season in *Unio crassus* ranged from 1 to 5. Geographic variation in brooding patterns has also been documented in several species including *E. complanata* (Heard 1998). In most cases, the variation was latitudinal, involving a greater number of broods, longer brooding season, or longer brooding periods in warmer climates. However, exceptions sometimes occur when a population in the middle of the species range exhibits an atypical pattern (Heard 1998).

Elliptio complanata is a relatively well-studied, wide-ranging species. Fertilization and the start of the brooding period takes place from late April to May in Michigan (Matteson 1948) and Wisconsin (Baker 1928), slightly earlier than we observed gravid individuals (May 2). However, our sampling interval of two weeks did not allow us to pinpoint a start of the brooding season later than April 17 but earlier than May 2. Also, our sample sizes earlier in the season were low. Although one would expect an earlier start to the reproductive season in a more southern and warmer river, year to year variation is known to occur in reproductive timing within a population (Matteson 1948, Heard 1998). Perhaps *E. complanata* reproduced later in 2008 than was typical.

The duration of the study did not permit investigation of a second brooding season in the fall. Autumnal brooding has been documented in several North American mussels, but most *Elliptio* species appear to brood in one continuous season in the spring and early summer, or the winter in some tropical areas (Heard 1998). Our data on the length of the brooding period were consistent with the generalization that tachytictic species brood for about one month. However, many individuals, particularly *Elliptio angustata*, completed a brood in a much shorter time frame.

Freshwater mussels clearly exhibit a variety of life history strategies, including variable brood lengths and numbers of broods. Much more data on a wider range of species and populations are needed to investigate geographic variation in brooding patterns within species and selection pressure for various reproductive strategies. We hope that our research will encourage investigators to take a more detailed approach to investigating life history strategies and incorporate mark-recapture techniques. This study had limitations imposed by occasional high water conditions that limited sampling and by relatively low abundance of most species. Mark-recapture sampling could be even more useful in gathering detailed life history data in densely populated areas in frequently sampled smaller streams.

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Molluscan community composition and richness in four high-elevation Idaho streams includes an exotic taxon

Steven J. Lysne[1,2], Gabriel Garcia[1], and Bryan R. Krouse[1]

[1] The College of Western Idaho, 5500 E. Opportunity Way, Nampa, Idaho 80749, U.S.A.

[2] Orma J. Smith Museum of Natural History, The College of Idaho, 2112 Cleveland Boulevard, Caldwell, Idaho 83605, U.S.A.

Correspondence, S. J. Lysne: stevelysne@cwidaho.cc

Abstract: Effective conservation requires that natural resource managers understand the diversity of organisms within a jurisdictional unit. Too frequently, conservation priorities are determined without adequate biological information, leading to inefficient use of very limited resources. We investigated molluscan community composition and richness within the eastern portion of the Sawtooth National Recreation Area (*i.e.*, management unit) in Custer County, Idaho, USA to (1) identify populations of non-native species, if present, (2) acquire baseline information on the distribution, relative abundance, and diversity of molluscs in this high-elevation ecosystem, and (3) provide resource managers with useful information with which to inform conservation priority-setting. We found a rich molluscan community comprised of 19 species from 11 genera and 7 families. We also documented a single population of the non-native species *Radix auricularia* Linnaeus, 1758 from samples at Alturas Lake. Management of the very popular, eastern portion of the Sawtooth National Recreation Area should include public outreach regarding invasive species, importance of proper cleaning of boats and fishing gear, and importance of native invertebrates to healthy, functioning ecosystems.

Key words: diversity, non-native, *Margaritifera*, headwater, Salmon River

Effective conservation of molluscs requires that natural resource managers understand the taxonomy of the species within their jurisdiction, the richness of taxa within a given area, and the status of those taxa over time (Lysne *et al.* 2008). In Idaho, freshwater molluscs have received considerable recent attention due to the endangered status of some species (Lysne and Koetsier 2006, Richards *et al.* 2006, Myler *et al.* 2007), descriptions of new species (Hershler *et al.* 2006), taxonomic revisions of others (Hershler and Liu 2004, Wethington and Lydeard 2007), concern with regard to non-native species (Richards *et al.* 2001, Lysne and Koetsier 2008, Hershler *et al.* 2010), and efforts to document the richness of species state-wide (Lysne and Clark 2009). Of particular concern to resources managers in Idaho is the presence and status of non-native freshwater mollusc invasions. Of the 117 putative freshwater mollusc taxa in Idaho, as many as 15 are non-native species (Frest and Johannes 2000) and others are likely to become established within the next decade (*e.g.*, species of *Dreissena* Beneden, 1835). Our study investigated four high-elevation lakes and tributaries to the upper Salmon River within the Sawtooth National Recreation Area (SNRA) to describe the freshwater molluscan community, estimate abundance, document the presence of non-native species, and determine the distribution of non-native species. The lakes and rivers of the SNRA are popular with recreationists and sportsmen, and the probability of invasions of exotics was considered to be high.

MATERIALS AND METHODS

Study area

The SNRA lies within the Sawtooth National Forest in south-central Idaho (Fig. 1) and is in the molluscan zoogeographical region known as the Western American Division (Burch 1989). Annual precipitation ranges between 51 and 112 cm over the period of record from 1978 to 2009 (http://www.wcc.nrcs.usda.gov/cgibin/snotel/). We limited our work to four high-elevation lakes, and their inlet and outlet creeks (hereafter known as "sites"), on the east side of the SNRA, south of the town of Stanley, Idaho. The sites—Alturas, Petitt, Stanley, and Redfish Lakes—range in elevation from 1,981 m to 2,133 m and are each fed by 1st order creeks originating from the Sawtooth Mountain Range. These tributaries to the Salmon River exhibit a classic snowmelt hydrograph (Allan 1995) characterized by high flows in May and June and low flows from late fall to mid-winter. We observed maximum mean summer temperatures between 13 and 25 °C and very low conductivities (data available on request to SJL). Daily mean air temperature is consistently below freezing from mid-October to mid-May (http://www.wcc.nrcs.usda.gov/nwcc/view), resulting in extensive ice cover annually and a relatively short growing season. Thus, each lake and creek is considered oligotrophic relative to waterbodies in Idaho at lower elevations.

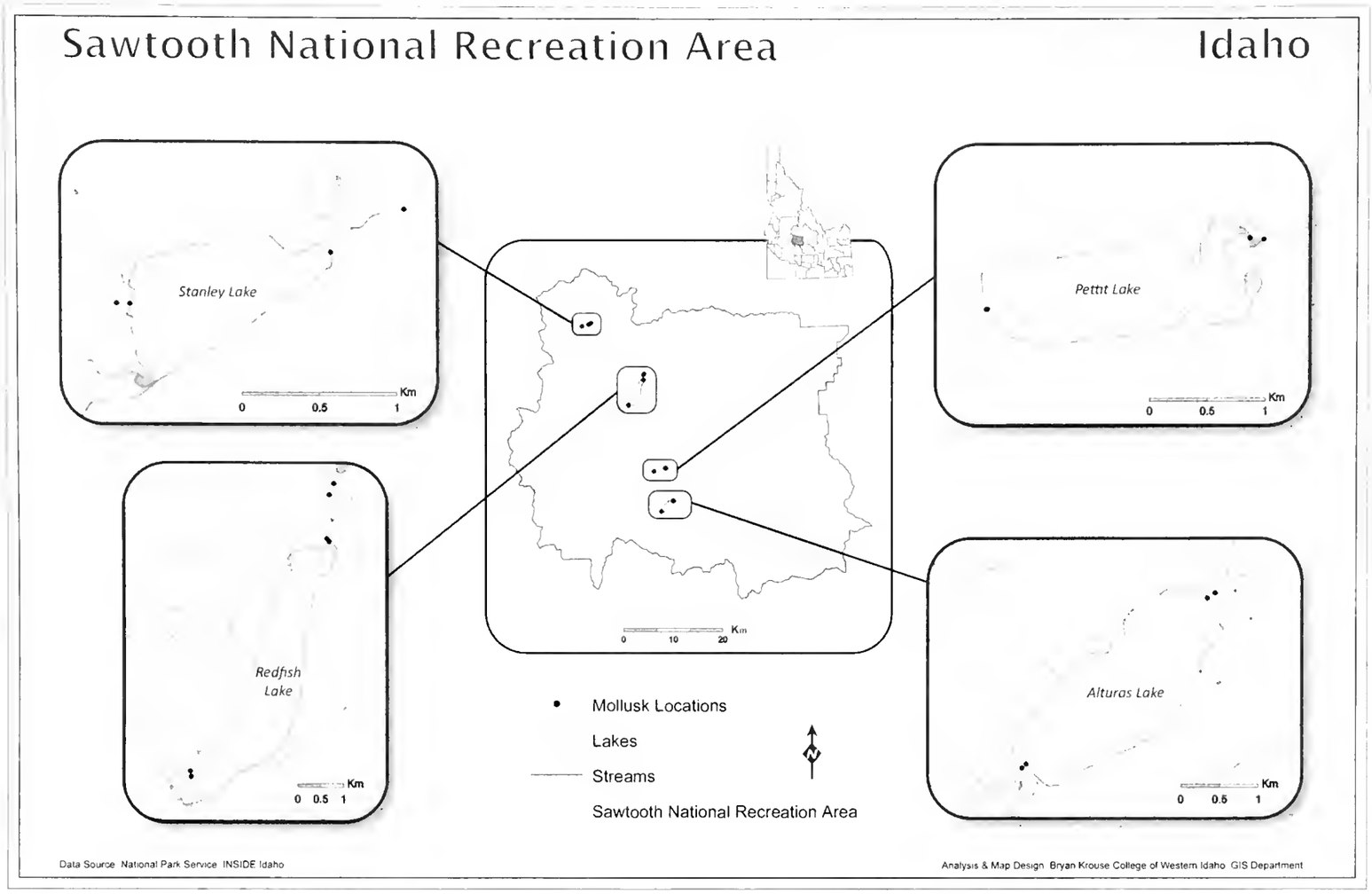

Figure 1. Map of the Sawtooth National Recreation Area showing the location of lakes surveyed and samples collected.

Field sampling

All sites were surveyed during the fall of 2005 by U.S. Forest Service biologists. Both qualitative (timed searches) and quantitative (systematic sampling with multiple random starts) methods were used to capitalize on the benefits of different survey techniques. Because habitat types in a survey location can be visited with reasonable effort, qualitative surveys can be useful for documenting species presence. Quantitative sampling, by contrast, is preferable to estimate abundance and associated variances (Strayer and Smith 2003) but may not detect rare species in micro-habitats missed by systematic sampling.

During qualitative surveys, biologists visually searched for molluscs using benthic viewers in wadeable portions of a lake or creek (*i.e.*, <1 m depth). In creeks, all habitat types were surveyed, non-randomly, within 300 m of the inlet and outlet of each lake for three person-hours. Methods were similar between sites but were limited in lakes to the wadeable shoreline on both sides of the inlet and outlet; survey time was one person-hour. Representative specimens of each unique taxa (with the exception of *Margaritifera falcata* Gould, 1850) were collected and preserved in 70% ETOH. Multiple specimens were collected for some taxa as all individuals could not be readily identified in the field. When *M. falcata* were encountered, researchers measured each individual to the nearest mm and returned the specimen to its place of origin. No attempt was made to collect all individuals observed, or to quantify species abundance or density via qualitative surveys. Qualitative surveys contributed to estimates of species richness only.

Biologists conducted quantitative, systematic sampling on the outlet creeks at Alturas, Redfish, and Stanley Lakes only. Due to resource limitations, Petitt L. was not included in systematic sampling and was thus excluded from all analyses based on systematic samples. A systematic survey design was used at each site following Strayer and Smith (2003). Systematic surveys are efficient for rare or clustered populations and multiple random starts help provide accurate estimates of variance (Christman 2000). Biologists delineated a 100 m reach of each creek as near the mouth of the outlet as

possible subject to safety concerns such as extreme flow velocities, stream gradients, and plunge pools. A grid was superimposed over the 100 m reach and coordinates were generated for at least three random starts. Subsequent systematic samples were collected at the y^{th} interval following Strayer and Smith (2003). Each random start and resulting samples constituted an experimental unit. Biologists sampled a minimum of 50 quadrats at each site using benthic viewers. Quadrats were constructed of PVC piping and were either 1 m^2 or 0.25 m^2 depending on the estimated density of molluscs determined during pilot studies conducted *a priori*. At Alturas and Redfish Lakes, researchers used a 1 m^2 quadrat and at Stanley L., where mollusc density was relatively high, a smaller, 0.25 m^2quadrat, was used. We acknowledge that the use of different quadrat sizes complicates our comparisons but believe that a simple scaling factor allows comparisons and violates no assumptions. Estimates of biomass and diversity are derived from quantitative sampling only.

For each quadrat, biologists visually inspected the benthos and removed any benthic substrate greater than *ca.* 30 mm in diameter for inspection of all surfaces. A quadrat was considered "surveyed" when all substrates >30 mm were removed and the quadrat had been thoroughly examined. Researchers did not use a suction dredge or excavate embedded cobbles or boulders, and not all individuals present at any given site were collected, thus our findings are likely to be underestimates for several taxa including the limpets and fingernail clams. All samples were preserved in 70% ETOH with the exception of *Margaritifera falcata* which were measured to the nearest mm and returned to their place of origin.

Laboratory processing

U.S. Forest Service biologists shipped the preserved specimens to the College of Western Idaho for enumeration, identification, analysis, and preparation for museum curation. All individuals of each taxon (with the exception of *Margaritifera falcata*) were grouped together after identification and weighed to the nearest 0.001 g. We used wet-weight for biomass calculations. Molluscs were removed from vials, blotted dry, and then weighed; we did not remove soft tissue from shells. For *M. falcata*, we used unpublished length/wet-weight regressions to estimate biomass. All samples are available for reference or further study at the Orma J. Smith Museum of Natural History (http://www.collegeofidaho.edu/campus/). All individuals from each site were identified following Burch (1989) and Wethington and Lydeard (2007) and nomenclature followed Turgeon *et al.* (1998).

Data analysis

We calculated a single species richness value for each of the four sites by enumerating unique taxa. We did not differentiate between richness in the lake versus richness at inlet or outlet creeks. We also calculated the Simpson's Diversity Index (D) for each site and compared these values descriptively across sites. We used only samples from the outlet creeks where systematic sampling was conducted for biomass and D comparisons, thus we did not use Petitt L. data in these analyses. Biomass (grams/m^2) for all taxa collected at each site was summed and compared across sites using one-way ANOVA. We multiplied biomass values for Stanley L. samples by a factor of four before comparisons were made due to the smaller quadrat size used in Stanley L. Creek (*i.e.*, 0.25 m^2 vs. 1 m^2) and our need to standardize sampling units for statistical purposes. We constructed species importance curves following Lysne and Clark (2009). Species importance curves represent dominant taxa based on abundance or, in our case, biomass and assume ecological importance based on that dominance. Curves in our study are based on biomass and were created for the entire SNRA and for each site then compared descriptively across sites.

RESULTS

We collected and identified 2,366 freshwater molluscs from 514 samples collected at four alpine lakes. Species richness at the SNRA consists of 19 species from 11 genera and 7 families (Table 1). Though only one population was observed, abundance of *Margaritifera falcata* dominated that molluscan fauna (Fig. 2). Biomass was not different within sites surveyed but differed significantly across sites ($\alpha = 0.05$; $df = 2, 187$; $P < 0.001$; Table 2). The greatest value for biomass was observed at Stanley L. where *M. falcata* was most abundant (Fig. 3) by two orders of magnitude. At Alturas and Redfish Lakes, the molluscan fauna was dominated by *Radix auricularia* Linnaeus, 1758 and *Stagnicola emarginata* Say, 1821, respectively (Fig. 3). Species richness was greatest at Stanley L. followed by Alturas and Redfish Lakes (Table 3). Species diversity was highest at Redfish L. ($D = 2.16$) relative to Stanley L. ($D = 3.36 \times 10^{-4}$) and Alturas L. ($D = 2.06 \times 10^{-4}$). We documented a single, extant population of non-native species in the SNRA. We collected 144 *R. auricularia* from 38 samples at Alturas L. We also collected shells of the non-native *Planorbella duryi* Wetherby, 1879 in Stanley L. but failed to document an extant population there.

DISCUSSION

We conducted a survey of four alpine lakes in the Sawtooth National Recreation Area (SNRA) to identify mollusc populations of management concern and to describe the mollusc community in this remote, high-elevation ecosystem. Understanding the diversity, distribution, and

Table 1. Molluscs present during sampling in the Sawtooth National Recreation Area, Custer County, Idaho. Data show presence related to lakes as well as conservation status. Voucher specimens of each taxon are kept at the Smith Museum of Natural History. Abbreviations: A, Alturas; P, Pettit; R, Redfish; S, Stanley. Rankings are from NatureServe (2010): G1/S1, critically imperiled; G2/S2, imperiled; G3/S3, vulnerable; G4/S4, apparently secure; G5/S5, secure; SNR, state not ranked; EXO, exotic/introduced; NA, no ranking available. *Collected as shell only.

Family	Genus	Species	Authority	Lake	G rank	S rank
Gastropods						
Ancylidae	*Ferrissia*	*rivularis*	Say, 1817	S	G5	SNR
Lymnaeidae	*Fossaria*	*obrussa**	Say, 1825	A	G5	SNR
Lymnaeidae	*Fossaria*	sp.	Westerlund, 1885	S	NA	NA
Lymnaeidae	*Pseudosuccinea*	*columella**	Say, 1817	A	G5	SNR
Lymnaeidae	*Radix*	*auricularia*	Linnaeus, 1758	A	G5	EXO
Lymnaeidae	*Stagnicola*	*emarginata*	Say, 1821	A,P,R	G5	NA
Physidae	*Physa*	*gyrina*	Say, 1821	A,P,R,S	G5	SNR
Planorbidae	*Gyraulus*	*circumstriatus*	Tryon, 1866	A,P,R,S	G5	SNR
Planorbidae	*Gyraulus*	*parvus*	Say, 1817	A,P,R,S	G5	SNR
Planorbidae	*Gyraulus*	*deflectus*	Say, 1824	S	G5	SNR
Planorbidae	*Planorbella*	*ammon*	Gould, 1855	S	NA	NA
Planorbidae	*Planorbella*	*duryi**	Wetherby, 1879	S	G5	EXO
Planorbidae	*Planorbella*	sp.	Haldeman, 1842	S	NA	NA
Planorbidae	*Planorbella*	*subcrenata*	Carpenter, 1857	S	G5	SNR
Valvatidae	*Valvata*	*humeralis**	Say, 1829	R,S	G5	SNR
Bivalves						
Margaritiferidae	*Margaritifera*	*falcata*	Gould, 1850	A,R,S	G4	SNR
Sphaeriidae	*Pisidium*	*casertanum*	Poli, 1791	A,P,R,S	G5	SNR
Sphaeriidae	*Pisidium*	*conventus*	Clessin, 1817	S	G5	SNR
Sphaeriidae	*Pisidium*	*insigne*	Gabb, 1868	S	G5	SNR

trend of biological communities is a prerequisite to setting conservations priorities (Lysne *et al.* 2008). Our understanding of the distribution and abundance of mollusc communities, including species of management concern, has benefitted in recent years from the efforts of scientists across North America (Lysne and Clark 2009, Dillon *et al.* 2010, Evans and Ray 2010).

Of primary interest are the potential presence, distribution, and abundance of non-native species within the SNRA management unit. The SNRA supports considerable recreational boating, and the spread of non-native species via recreational boaters is of concern. The state of Idaho has recently increased awareness and enforcement of nuisance aquatic species violations targeting specifically members of the freshwater genus *Dreissena* [http://www.idahoag.us/Categories/Environment/InvasiveSpeciesCouncil/index]. Our survey of the SNRA, fortunately, failed to find any non-native bivalve molluscs (*e.g.*, *Dreissena* sp. or *Corbicula* sp. von Muhlfeld, 1811) but did document an extant population of *Radix auricularia* in Alturas L. This non-native gastropod was, in fact, the most abundant member of the molluscan community at Alturas L. While *R. auricularia* populations are not uncommon in Idaho, the relative abundance of this species in Alturas L. should be of concern to resources managers. It is of ecological interest, however, that *R. auricularia* was found only in one site.

Also of interest is that certain taxa were found exclusively at either Stanley L. in the north of the SNRA or at Alturas L. in the south of the SNRA. Neither Redfish nor Pettit Lakes contained any taxa that were unique to those systems. This suggests two molluscan faunas, one in the north and one in the south of the SNRA, mixing together in middle-latitude sites. From our collections, species belonging to the family Planorbidae Rafinesque, 1815 are distributed in the northern portions of the SNRA and those of the family Lymnaeidae Rafinesque, 1815 are distributed primarily in the south. Elucidation of this distribution difference will await a more detailed study.

A single population of *Margaritifera falcata* was observed in Stanley L. Creek. A single individual *M. falcata* was observed at both Alturas and Redfish Lakes during qualitative sampling, but was not picked up during systematic sampling. Mussel beds frequently display highly clumped distributions (Strayer and Smith 2003), so it is possible that active beds were missed due to our systematic sampling design. Indeed, beds have been observed downstream of the experimental reach in both Redfish and Alturas L. Creeks (SJL, pers. obs.). Is it because of *M. falcata* that molluscan biomass in Stanley L. is nearly two

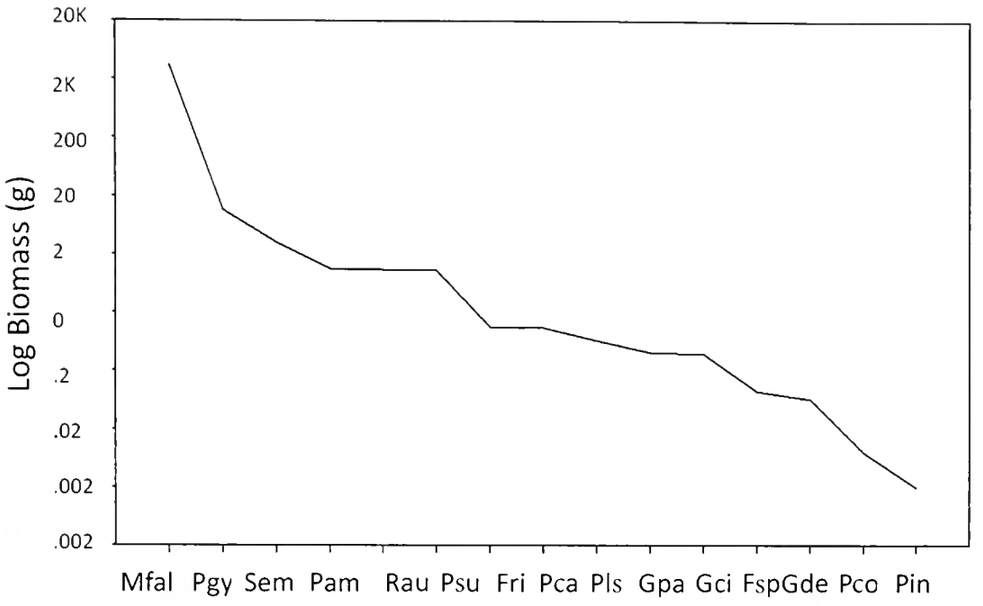

Figure 2. Species importance curve for molluscs in the SNRA. Abbreviations are: **M**argaritifera **fal**cata, **P**hysa **gy**rina, **S**tagnicola **em**arginata, **P**lanorbella **am**mon, **R**adix **au**ricularia, **P**lanorbella **su**bcrenata, **F**errissia **ri**vularis, **P**isidium **ca**sertanum, **P**lanorbella sp., **G**yraulus **pa**rvus, **G**yraulus **ci**rcumstriatus, **F**ossaria **sp.**, **G**yraulus **de**flectus, **P**isidium **co**nventus, and **P**isidium **in**signe.

Table 2. Analysis of variance of mean biomass for molluscs within and between sites. No significant differences were detected within the three sites but differences between sites were significant at $\alpha = 0.05$.

Site	*df*	*F*	*P*-value
Within groups			
Alturas Lake	142	3.06	0.09
Redfish Lake	78	3.11	0.64
Stanley Lake	244	2.25	0.06
Between groups	2	3.01	< 0.001

orders of magnitude greater than in either Alturas or Redfish Lakes? Does the presence of abundant *M. falcata* also explain why species richness is nearly double at Stanley L. relative to all other sites in our study? Vaughn and Spooner (2006) demonstrated that macroinvertebrate densities are significantly greater in stream segments where mussels are found relative to stream segments without mussels. They suggest that nutrient enrichment via biodeposits, or pseudofeces, support greater productivity in mussel beds and thus influence macroinvertebrate assemblages. Our results are consistent with their conclusions and demonstrate greater species richness and biomass, even when excluding mussel biomass, in streams with mussels compared to without.

We were surprised to find this high-elevation ecosystem is so speciose. Clarke *et al.* (2008) reviewed world-wide studies of macroinvertebrate richness and generally found that richness was greater in mid-order streams. Vannote *et al.* (1980), of course, proposed the River Continuum Concept around this phenomenon. The streams in our study, however, were all 1st or 2nd order and at high elevations. Similar work in Idaho has found comparable values for molluscan richness (Lysne and Clark 2009) but the composition of species, the habitat, and stream order differed greatly. For example, three molluscan families contributed disproportionately to the high richness value for the SNRA—Lymnaeidae, Planorbidae, and Sphaeriidae Deshayes, 1855—and the Lymnaeidae were the most important in two of four sites (Figs. 5, 6). Most studies of species richness, however, emphasize EPT taxa (*i.e.*, the orders Ephemoptera, Plecoptera, and Trichoptera), and if molluscs are included at all, they are determined only to the taxonomic level of class. Hieber *et al.* (2005) investigated community composition in alpine streams in Finland and found that collector/gatherers dominated the outlets of alpine lakes. This study too considered primarily EPT taxa; only a single mollusc was reported in all collections (Hieber *et al.* 2005; Appendix 1). However, we can compare their information on functional feeding groups to our molluscan assemblage. In our study, sites were dominated by scrapers, not collector/gatherers, with the exception of Stanley Lake where the filter-feeding *Margaritifera falcata* dominated the community. Harding (1994) conducted a much broader study of community composition in high-elevation lakes in New Zealand. While his work provides the best information for comparisons of richness, it is a very different ecological system, but with similar abiotic parameters. Harding investigated 20 alpine lake outlets and found that richness was significantly lower at the highest elevation lakes. He also found that the functional feeding groups at higher elevation sites were dominated by collector/gatherers and filter feeders. Molluscs were not abundant in alpine lakes in his study until lower elevations, *ca.* 200 to 500 meters (Harding 1994). Our results differ from both Hieber *et al.* (2005) and Harding (1994) and are interesting because, although we did not sample for all stream taxa, we found a diverse community of filterer and scraper functional feeding groups.

In summary, the Sawtooth National Recreation Area has a relatively rich molluscan community including a large population of *Margaritifera falcata* and at least one viable population of non-native molluscs. There was no evidence of the highly invasive *Potamopyrgus antipodarum* Gray, 1843 or

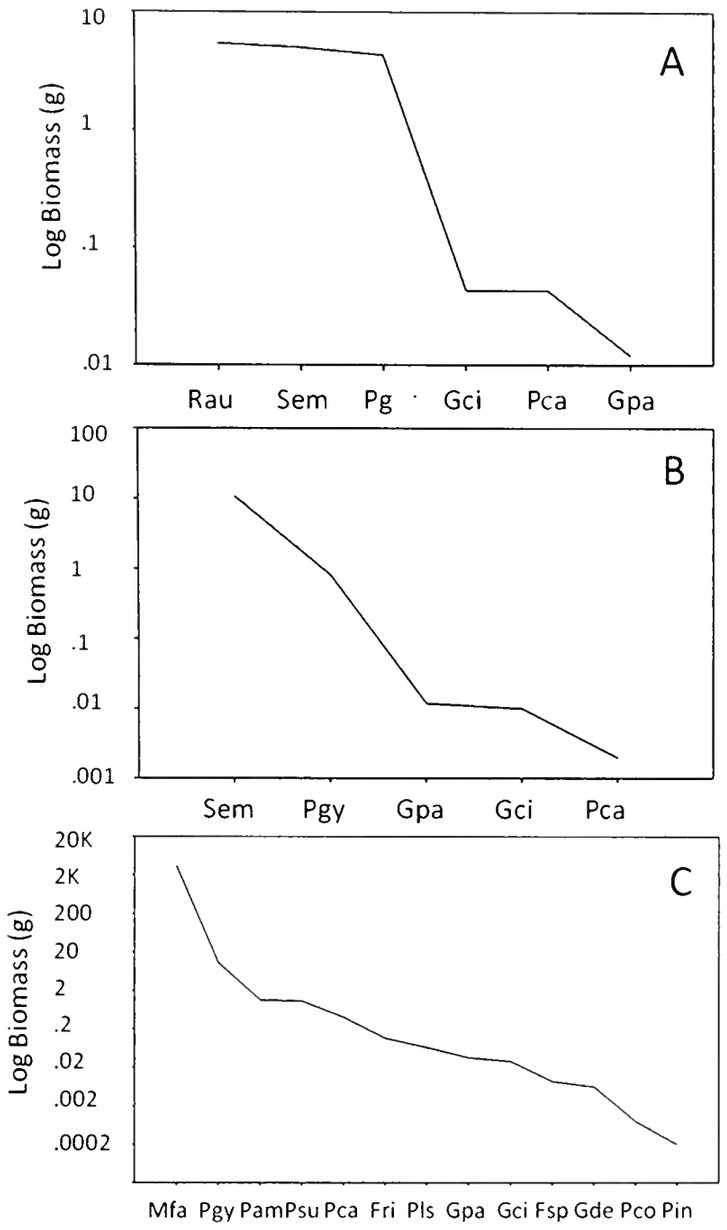

Figure 3. Species importance curve for molluscs collected live in Alturas (A), Redfish (B), and Stanley L. (C) sites. Abbreviations are: Ferrissia **ri**vularis, **F**ossaria **sp.**, Gyraulus **c**ircumstriatus, Gyraulus **de**flectus, Gyraulus **par**vus, **M**argaritifera **fal**cata, Physa **gy**rina, Pisidium **con**ventus, Pisidium **in**signe, **P**lanorbella **sp.**, Planorbella **am**mon, Planorbella **su**bcrenata, Pisidium **ca**sertanum, **R**adix **au**ricularia, and Stagnicola **em**arginata.

Dreissena sp. Natural resource managers, however, should monitor the population of *Radix auricularia* at Alturas L. and work to prevent the spread of this, and other, nuisance species to alpine lakes in this relatively pristine ecosystem. Management should also include public outreach regarding invasive species, the importance of properly cleaning boats and fishing gear, and the importance of native invertebrates to a healthy, functioning ecosystem. Finally, the distribution of *M. falcata* in the SNRA is uncertain and we suggest future workers learn why this species is apparently restricted to Stanley L. Creek or, alternatively, why it exists at such low densities as to avoid detection at Alturas and Redfish L. Creeks.

Table 3. Molluscan species richness, diversity (Simpson's *D*), and estimated biomass (g/m^2) at each site compared to lake area. Notice that biomass and diversity values for Pettit Lake are excluded (NA); see methods for explanation.

Location	Lake area (km^2)	Biomass (g/m^2)	Richness	Diversity
SNRA		35	19	3.41
Alturas Lake	3.4	0.097	9	$2.06*10^{-4}$
Redfish Lake	6.1	0.129	7	2.16
Stanley Lake	0.7	68.54	15	$3.36*10^{-4}$
Pettit Lake	1.6	NA	5	NA

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RESEARCH NOTE

Invasion of the Argentinean Paranense rainforest by the giant African snail *Achatina fulica*

Diego E. Gutiérrez Gregoric, Verónica Núñez, Roberto Vogler, and Alejandra Rumi

CONICET, Invertebrate Zoology Division, La Plata Museum (MLP), National University of La Plata, La Plata, Paseo del Bosque s/n (B1900FWA), Argentina

Correspondence, D. E. Gutiérrez Gregoric: dieguty@fcnym.unlp.edu.ar

Abstract: The tropical land snail *Achatina fulica* Bowdich, 1822, native to Africa, is reported for the first time in Argentina, in Puerto Iguazú City, Misiones Province. This city is surrounded by protected areas. Three randomly selected 1-m^2 plots were marked off in private gardens and in the area surrounding an urban stream for snail sampling. The high snail density detected could have ecological, sanitary, and economic consequences which have already been documented in other countries.

Key words: invasive land mollusc, Misiones, Argentina, impact

The giant African snail *Achatina fulica* Bowdich, 1822 is a tropical land snail native to East Africa that is considered as one of the world's most damaging pests (Lowe *et al.* 2000). In part, this is due to the introduction to several countries and traits that increase its invasion ability (polyphagous diet, adaptability, and dispersion). *Achatina fulica* was introduced into Brazil from Indonesia, in the 1980s for unsuccessful commercial purposes. Now, it is widespread in almost all of this country (Thiengo *et al.* 2007). In Argentina, at the present, 21 alien land gastropods have been recorded (Rumi *et al.* 2010), but the presence of *A. fulica* was unknown.

This note reports the introduction of *Achatina fulica* in Argentinean territory at Puerto Iguazú City (25°36′S, 54°35′W). This city is located in the extreme northwestern corner of Misiones Province, Argentina, at the border with Brazil and Paraguay. It is surrounded by protected areas such as the Iguazú National Park (676.2 km^2), Puerto Península Provincial Park (69 km^2), and Urugua-í Provincial Park (840 km^2) in Argentinean territory.

Individuals of *Achatina fulica* were collected in March 2010 (MLP N°13185). Sampling took place during day, with a temperature of 31 °C and humidity of 70%. Snails were collected from soil within three 1-m^2 plots randomly distributed in the study area. Two of them were located in private ornamental gardens, one with decomposing branches and creeping vegetation, and the other one below a banana tree. The third plot was located beside Mariposa stream (an urban stream). Specimens of *A. fulica* were classified in four size classes: recently hatched (up to 10 mm shell length), juvenile (10 to 40 mm), young adults (40 to 70 mm), and adults (>70 mm) (Simião and Fischer 2004). Densities of *A. fulica* and native gastropods per plot were calculated.

All size classes of *A. fulica* were found from eggs and newly-hatched juveniles to adult snails up to 110 mm in height.

Mean density was 107.6 snails/m^2 for *Achatina fulica* (range: 10 to 186) and 6 snails/m^2 for native gastropods (range: 1 to 10) (Table 1). Native gastropods belonged to Scolodontidae (*Happia* Bourguignat, 1889), Streptaxidae (*Rectartemon* Baker, 1925), and Orthalicidae (*Cyclodontina guarani* (d'Orbigny, 1835)) (Table 1). Other specimens of *A. fulica* were on trees, trunks, posts, walls, and roofs of houses. In spite of being nocturnal, individuals were found active during the day.

Reasons for the widespread introduction of *Achatina fulica* are numerous (Cowie and Robinson 2003). Although it is not possible to determine the vector, the introduction of this species in Argentina is probably linked to fishing. The use of snails as fish bait is a frequent practice and *A. fulica* from Brazil may have been accidentally released by fishermen into riversides of the Paraná and Iguazú Rivers (next to Puerto Iguazú City). Densities for *A. fulica* suggest a stable population. Residents reported that the species appeared approximately three years ago.

In the newly invaded area (Misiones Province), 25 native species of land gastropods occur: Charopidae (2 species), Euconulidae (1), Gastrodontidae (1), Helicinidae (3), Orthalicidae (5), Pupillidae (1), Scolodontidae (2), Solaropsidae (1), Streptaxidae (2), Strophocheilidae (3), Succineidae (1), and Veronicellidae (3) (Fernández 1973). The presence of *Achatina fulica* may pose a threat to native terrestrial molluscs through competition for food and refuge (Fischer and Colley

Table 1. Density (number/m^2) of *Achatina fulica* and native gastropods in Puerto Iguazú city, Argentina.

	Habitat		
Taxa	Garden	Garden banana tree	Stream
Achatina fulica			
Recently hatched	171	33	0
Juvenile	10	93	0
Young adults	1	0	6
Adults	4	1	4
Total	**186**	**127**	**10**
Native Gastropods			
Rectartemon sp.	7	8	0
Happia sp.	0	2	0
Cyclodontina guarani	0	0	1
Total	**7**	**10**	**1**

2004). Furthermore, this species is known to be a snail predator (Meyer *et al.* 2008).

Creeping vegetation that constitutes refuge for micromolluscs (Scolodontidae and Charopidae) is consumed by the invasive species. The native snail *Megalobulimus sanctipaulis* (Ihering and Pilsbry, 1900) and *M. oblongus* (Müller, 1774), that superficially resemble *Achatina fulica*, may be vulnerable to competition with *A. fulica*, especially because they lay only a few eggs (Jurberg *et al.* 1988). The most effective control method for *A. fulica* is manual capture and destruction of specimens. This fact represents a risk for *Megalobulimus* species as uninformed people may confuse natives with invasive species (Cuezzo 2004).

Achatina fulica acts as an intermediate host of several parasites and may spread abdominal angiostrongyliasis (etiological agent: the nematode *Angiostrongylus costaricensis*) and the establishment of *A. cantonensis* and others diseases in Argentina (Caldeira *et al.* 2007).

Besides environmental and human health costs caused by this introduced species, economic losses may occur from the devastation of more than 100 crops and stored grains, and it is important to take immediate control measures. For example, in Misiones Province there are 263.8 km^2 of tobacco (*Nicotiana tabacum*), 348.99 km^2 of tea (*Camellia sinensis*) and 78.51 km^2 of cassava (*Manihot* spp.) plantations, which represent the 40%, 95%, and 97% of national production respectively (National Institute of Statistics and Censuses 2002). These plantations are susceptible to *Achatina fulica* (Raut and Barker 2002, Cuezzo 2004). Other plantations in the area, like the 1677.22 km^2 of mate (*Ilex paraguariensis*) that represent the 92% of national production, could be also affected. Control measures, preventive education, and surveillance are needed to prevent its further spread. However, it is important that control efforts, for instance using pesticides or physical/mechanical methods, do not affect the native species.

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RESEARCH NOTE

Wayne Grimm's legacy: A 40-year experiment on the dispersal of *Cepaea nemoralis* in Frederick County, Maryland

Aydin Örstan[1], James L. Sparks Jr.[2], and Timothy A. Pearce[1]

[1]Section of Mollusks, Carnegie Museum of Natural History, 4400 Forbes Ave., Pittsburgh, Pennsylvania 15213-4080, U.S.A.

[2]Wildlife and Conservation Ecology, Appalachian Laboratory, 301 Braddock Rd., Frostburg, Maryland 21532, U.S.A.

Correspondence, A. Örstan: pulmonate@earthlink.net

Key words: land snail, introduced species, colonization

The American-Canadian malacologist F. Wayne Grimm (1941-2005) started surveying the land snails of Maryland and Virginia in the 1950s and continued until his emigration to Canada in 1969. In 1977, Grimm's malacological activities went into a long hiatus as he turned to other pursuits. The revival of his interest in gastropods in the early 1990s ended when Grimm died from complications of diabetes in 2005 (F. Schueler, pers. comm.).

In a recapitulation of the surveys he had done in Maryland, Grimm (1971: 51) listed more than 100 terrestrial gastropod taxa, including the European snail *Cepaea nemoralis* (Linnaeus, 1758) with this annotation: "Deliberately introduced to Frederick Co. from Warm Springs, Va., in July, 1969." Grimm revealed neither the exact location of this introduction, nor the identity of the responsible person, although he himself was a likely culprit as he had earlier introduced *C. nemoralis* to the yard of his house in Baltimore (Reed 1964). A clue to the whereabouts of the introduced *C. nemoralis* emerged 24 years later when Counts and Weissman (1993: 22) reported finding a *C. nemoralis* shell that year in Frederick County "approximately 200 m north of Route 40." To our knowledge, no other record of *C. nemoralis* from Frederick County has been published since 1971.

In 2002, one of us (TP) e-mailed Grimm for more information about the locality of *Cepaea nemoralis* in Frederick County. Grimm responded with a phone message. The relevant parts of the message, transcribed by TP and e-mailed to AÖ in May 2002, were: "The place in Frederick County where the snails were introduced is the old Monocacy River bridge, where US 40 crossed it at one time. I went there in 1996 and found every trace of the bridge gone, and all the weeds around it were gone, too, so I strongly suspect that the snails are no longer there, but it might be a valid thing to take a look at them. Again, the old Monocacy River bridge in Frederick Co., which was the old US40 bridge, right at the weedy spot that was there back in the 60s, actually the 50s. I introduced them quite a long time ago."

Although Grimm had revealed the missing pieces of the puzzle, our intention in 2002 to search for Grimm's *Cepaea nemoralis* was soon forgotten. Then in May 2009—almost exactly 40 years after Grimm's release of *C. nemoralis*—JS came upon large, colorful snails near a highway culvert in Frederick County. He e-mailed their pictures to AÖ who identified the snails as *C. nemoralis*. This find rekindled our interest in Grimm's snails and initiated a survey that has delineated their current range.

Our survey took place between May 2009 and January 2010. We searched for snails at 79 stations, coded FR-2 through FR-80, around Grimm's point of introduction on both sides of the Monocacy River (Fig. 1). We obtained GPS coordinates of each station and corrected them when necessary using Google Earth. We measured the maximum dispersal distances using Google Earth along straight lines between the point of introduction and the furthermost stations where *Cepaea nemoralis* was found. To estimate annual dispersal rates, we divided maximum dispersal distances by 40 years. A list of stations and coordinates is available from the authors. Voucher specimens have been deposited in the Carnegie Museum of Natural History (CM102302-102303, 102856-102857, 102859, 102863-102865, 102867-102870, 102877, 102888).

Presently, three bridges cross the Monocacy River east of the city of Frederick (Fig. 1). The northern and newest bridge, built in the late 1980s, is for US40 and I-70. Approximately 600 m south are two other bridges. The one built in 1954 currently handles both westbound and eastbound traffic on MD144; but prior to 1990 carried only eastbound traffic. The adjacent bridge, built in 1942, carried westbound traffic until

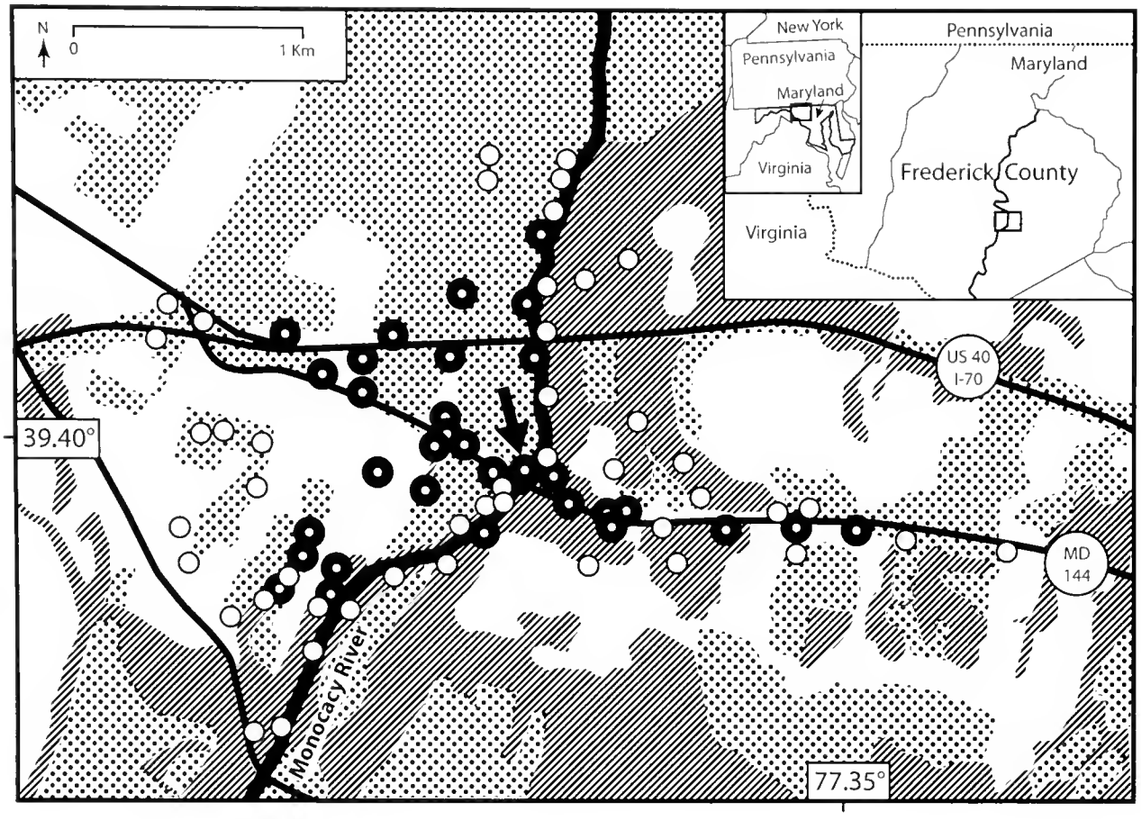

Figure 1. Map of survey area showing localities where *Cepaea nemoralis* was present (closed circles with open centers) and absent (open circles). The arrow marks the site inferred as Wayne Grimm's point of introduction. Land cover is forests (hatched), farm fields (stippled), and developed areas (white). The river in the survey area is approx. 50 m wide and flows south.

1990 when it was decommissioned (Nora Bucke, Maryland State Highway Administration, pers. comm.). According to the USGS 1:100,000-scale topographic map dated 1984 (39077-A1-TM-100), prior to the building of the newest bridge to the north, MD144 also carried the designations US40 and I-70. The west bank of the river has been used for farming and currently has farm fields as well as plots overgrown with weeds, while the east bank has retained more forest cover. Therefore, we infer that Grimm's point of introduction of *Cepaea nemoralis*, which he described as the "the old Monocacy River bridge in Frederick Co., which was the old US40 bridge, right at the weedy spot," was on the west bank of the Monocacy River immediately to the north of the now decommissioned bridge. For measurement of dispersal distances, we arbitrarily designated one of our localities on the west bank closest to the decommissioned bridge (station FR-4; 39.39878°N, 77.36699°W) as the point of introduction (arrow in Fig. 1). As to why Grimm could not locate his point of introduction in 1996, we speculate that he was not aware of changes in road numbers since 1969, and perhaps looked for the "old bridge" in the location of the present US40/I-70 bridge.

Our results show that dispersal of *Cepaea nemoralis* has taken place primarily to the west of the Monocacy River since 1969 (Fig. 1), supporting placement of Grimm's point of introduction on the west bank. The distribution of localities with *C. nemoralis* west of the river is consistent with radiating dispersal starting from one point. Also, the snails dispersed farther upstream than downstream, suggesting that transport by the river has not been important in their dispersal. In addition, a second and much narrower wave of dispersal has extended eastward across the river, mainly along MD144 (Fig. 1). We speculate that snails either crossed actively using the decommissioned bridge or were transported passively attached to motor vehicles travelling from west to east. Motor vehicles are implicated in dispersing snails eastward along MD144, because all occurrences more than about 400 m east of the river are on the south side of that road. Since traffic moves on the right side of the road in North America, a motor vehicle traveling eastward is likely to drop a snail on the right, on the south side of the road.

The habitats of *Cepaea nemoralis* in Europe include woods, hedges, grassland, roadsides, and railroad banks (Boycott 1934, Kerney and Cameron 1979). North American habitats of introduced *C. nemoralis* include mostly disturbed locations, such as university campuses, parks, residential gardens, fields, river banks, railroads, and roadsides (Reed 1964, Dundee 1974, Forsyth 1999, Schueler 2008, Örstan 2010). The broad habitat tolerances of *C. nemoralis* suggest that habitats in our survey area, which included mostly deciduous forest remnants bordering the Monocacy River, hedgerows, farm fields, roadside ditches, and culverts, would be suitable for the species. However, population densities of *C. nemoralis* among habitats were highly variable. For example, at station FR-38, the corner of a corn field separated from highway I-70 by a hedgerow and approx. 890 m northwest of the point of introduction, we collected approx.

110 shells in an area roughly 10 × 10 m. In contrast, at station FR-71, a forest remnant approx. 948 m southwest of the point of introduction, a search area that was also approx. 10 × 10 m yielded only three shells. Also, at several localities, which seemed suitable habitats for *C. nemoralis*, we could not find them. Some of those localities were between others where *C. nemoralis* was present.

Lamotte (1959) described the general distribution of *Cepaea nemoralis* in Europe as patchy colonies occupying limited areas with population densities from one to several hundred. Likewise, Wolda (1969) showed that the densities of *C. nemoralis* along an approx. 1.3-km road varied from one to 66 snails/m. According to Lamotte (1959), the distribution of the species was not continuous because suitable habitats were not continuous, and only a few isolated individuals, forming "migration trails," could be found between colonies.

The patchy distribution and variable densities of *Cepaea nemoralis* in our survey area agree with Lamotte's (1959) model. Presumably, the fastest dispersing individuals form the "migration trails" that cross the less suitable habitats in the shortest possible time. Some of those snails may establish new colonies if and when they reach suitable habitats, which then become new hubs for further dispersal. However, unlike Lamotte's more natural settings where plant cover may have determined habitat suitability, in our area habitat discontinuities arose mostly from humans: roads, buildings, parking lots, etc. Baur and Baur (1990) showed that an 8-meter wide paved road impeded the dispersal of *Arianta arbustorum* (Linnaeus, 1758), a snail comparable in size to *C. nemoralis*, during one 3-month activity season. They also observed that *A. arbustorum* preferred to disperse under the plant cover along roads. Wolda (1969), however, noted that *C. nemoralis* did cross roads. In our survey area, we often found *C. nemoralis* in ditches or hedgerows along roads, indicating that snails can use such suitable habitats to disperse along roads even if they cannot cross them often. The presence of several roads parallel with ditches in the east to west direction may explain the successful dispersal of *C. nemoralis* along the same directions in our survey area (Fig. 1). Also, culverts under many of the roads may have provided passageways for snails. Finally, in 40 years, successful crossing of a road even by a few snails could have founded a new colony on the other side.

The estimated maximum dispersal distances and annual dispersal rates of *Cepaea nemoralis* were: north along the west bank of the river, 1,097 m (27 m/y); west along MD144, 1,253 m (31 m/y); southwest 1,222 m (31 m/y); east along MD144, 1,534 m (38 m/y). These annual dispersal rates are within the range of published maximum active dispersal rates during six months or more for *C. nemoralis* and other land snails of comparable size. Some of the reported maximum dispersal rates for *C. nemoralis* are 46 m during six months and 67 m in two years (Schnetter 1951) and approx. 16 m in one year (Goodhart 1962). In addition, Cameron (2001) estimated about 10 m per year over more than 60 years for the dispersal of *C. nemoralis* in what he considered was a hostile environment of acidic soil and unstable habitats. Reported maximum dispersal rates for other land snails include 31 m in one year and 25 m in two years for *Helicigona lapicida* (Linnaeus, 1758), a species slightly smaller than *C. nemoralis* (Baur and Baur 2006); 42 m in six months for *Xeropicta derbentina* (Krynicki, 1836), also slightly smaller than *C. nemoralis* (Aubry *et al.* 2006); and 16 m in one year and 15 m in 10 months for *Arianta arbustorum*, a species about the size of *C. nemoralis* (Baur and Baur 1993).

Starting from a single introduction in 1969, the alien *Cepaea nemoralis* has dispersed far and wide in 40 years, and it would not be practical anymore to attempt to eradicate it. Luckily, the species does not appear to have become a pest. *Cepaea nemoralis* will probably continue to widen its range in Frederick County. We may never know what motivated Grimm to start this introduction, but his deed, although condemned in principle, will continue to provide data on snail dispersal for many years to come.

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Symposium on "Speciation in Molluscs" at the 75th Annual AMS Meeting

Warren D. Allmon

Paleontological Research Institution and Department of Earth and Atmospheric Sciences, Cornell University, Ithaca, New York 14850, U.S.A.

Correspondence, W. D. Allmon: wda1@cornell.edu

This issue of the *American Malacological Bulletin* contains six papers from a symposium on "Speciation in Molluscs", held at the 75th annual meeting of the American Malacological Society in Ithaca, New York on 20 July 2009 (Allmon *et al.* 2009). A total of nine papers were presented in the symposium, covering topics from chitons to nudibranchs. The six papers included here represent a considerable portion of this diversity of subject and approach (more than might be suggested by the fact that five of them are about gastropods!).

Rebecca Rundell critically reviews the remarkable life and career of John Thomas Gulick, who was one of malacology's most important early students of speciation. She notes that although modern research has altered some of Gulick's conclusions, we still puzzle as he did about the degree to which natural selection and geographic isolation contribute to species formation. Rob Dillon and John Robinson consider the topic of genetic differentiation *without* (or with only incomplete) speciation, focusing on the maddeningly complex systematics of the freshwater gastropod genus *Goniobasis/Elimia/Pleurocera* in the southern Appalachian region. Patrick Krug presents a review of the literature on speciation in modern marine gastropods and concludes that, counter to what is perhaps the received wisdom, there are abundant cases of non-allopatric speciation. Matthias Glaubrecht presents a wide-ranging review of speciation in modern freshwater gastropods. He concludes that although there is considerable evidence for various forms of sympatric speciation, allopatric speciation should still be used as the "default setting, or null-hypothesis for testing and falsification of non-allopatry by studying ecological and other relevant factors". In the only non-gastropod paper, Paula Mikkelsen surveys the subject of speciation in marine bivalves based on three sets of data from the literature. She reports that new species are still being described at a high rate, mostly from newly-collected material, and that many of these species were produced by relatively subtle processes, such as physiology, rather than (or in addition to) large-scale allopatry. Finally, Ursula Smith and I consider what, and how, the fossil record can tell us about speciation in marine gastropods. Writing this paper taught me a lot about what we still do not know – about both modern and fossil gastropods. We similarly find abundant "subtle" cases of speciation (*i.e.*, cryptic species), and ponder what this and other neontological data mean for our ability to consider causes of speciation using fossils, given the nature of the geological record.

Taken together, these six papers well illustrate some basic facts about the current state of our understanding of speciation: (1) allopatry still rules, but not by much; (2) cryptic species are very numerous and getting more so; (3) marine diversity may be higher than we think; and (4) we know a lot more about speciation than we used to, but not as much as many of us act like we do.

I am most grateful to the American Malacological Society for allowing me the opportunity to host the meeting in Ithaca and to bring together such a distinguished group of contributors to talk about one of my favorite subjects. I am also grateful to Cornell University's Department of Earth and Atmospheric Sciences for co-hosting the meeting with the Paleontological Research Institution. The meeting would not have materialized without the hard work of Kelly Cronin and the constant advice and support of Paula Mikkelsen, to both of whom I am most indebted. Ken Brown and Cynthia Trowbridge were enormously patient in waiting for and editing the manuscripts, and Maya Weltman-Fahs was enormously helpful in pulling all of them together at the end. Finally, I thank the authors who, despite several lengthy delays on my part, produced excellent and interesting manuscripts.

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Snails on an evolutionary tree: Gulick, speciation, and isolation*

Rebecca J. Rundell

Biodiversity Research Centre and Departments of Zoology and Botany, University of British Columbia, Canadian Institute for Advanced Research, Program in Integrated Microbial Biodiversity, Vancouver, British Columbia V6T 1Z4, Canada

Correspondence, R. J. Rundell: rrundell@interchange.ubc.ca

Abstract: Geographical separation is arguably fundamental to speciation. John Thomas Gulick (13 March 1832 – 14 April 1923), a missionary from the Hawaiian Islands and one of the earliest evolutionary biologists, was among the first to recognize the critical role for geographical separation in the diversification of ecologically similar Hawaiian land snails. Although Gulick's work is not well-known today, his ideas were discussed by Darwin and Wallace as well as leaders in the Modern Evolutionary Synthesis (*e.g.*, Wright and Mayr) who saw an important role for geographical isolation in speciation. It was perhaps no accident that organisms with low vagility, such as land snails of the Hawaiian Islands (*i.e.*, achatinelline tree snails and ground-dwelling amastrid snails) exemplified the importance of geographical separation in speciation. Here I provide context for Gulick's snail research, showing that the natural setting of the Hawaiian Islands, combined with Gulick's development as a naturalist and evolutionary thinker lead to important insights on speciation, resulting from observations of substantial species richness in achatinelline and amastrid land snails, among the ridges and valleys of the Hawaiian Islands. Gulick's research on lesser-known organisms, island land snails, illustrates key areas for future inquiry, particularly in understanding "nonadaptive" contributions to evolutionary radiations.

Key words: nonadaptive radiation, geographical, allopatric speciation, Hawaii

Many island land snail faunas exhibit high species richness in a very small land area (Crampton 1916, 1925, 1932, Solem 1990, Cowie *et al.* 1995, Cook 1996, 2008, Cowie 1996, Chiba 2004, Holland and Hadfield 2004, Parent and Crespi 2006, Rundell 2008, 2010). Examples of species with striking ecological differences can provide explanations for such diversity, but species with subtle or nonexistent ecological differences have been, and are, less well understood. Many closely related and morphologically similar species in other taxa (*e.g.*, birds) once suspected to be ecologically similar, were later found to be ecologically distinct (MacArthur 1958). Still species remain for which ecological differences are elusive (*e.g.*, snails; Gulick 1873a, 1889a). John Thomas Gulick (13 March 1832 – 14 April 1923), an evolutionist and missionary, was the discoverer of intra-island endemism among Hawaiian land snails (Gulick 1853, Reif 1985). He was unable to find ecological causes for the substantial species richness he observed in the Pacific endemic hermaphroditic pulmonate land snail families Amastridae and Achatinellidae (particularly subfamily Achatinellinae) within the Hawaiian Islands. Gulick used observations of these snails to develop theories on the role of geographical isolation in speciation, which were revolutionary in Darwin's time (Carson 1987, Hall 2006a).

Darwin's contemporaries, in the face of the new theory of natural selection, were perplexed by the vast array of morphological differences among species with no apparent adaptive significance; indeed, even much later such species-level variation proved confusing (Provine 1986: 453). Some argued for a critical role of natural selection, even for such unexplained differences (Wallace 1888, 1889), whereas other evolutionists sought explanations beyond natural selection (*e.g.*, "physiological selection" of Romanes: Provine 1986: 216). Those in the latter camp were criticized, particularly by naturalists, who eventually not only faced assault by Lamarckian views but also from the growing contingent of experimental biologists, whose work proliferated following the revelation of Mendel's genetic research in 1900. In its historical context (*e.g.*, given the widespread acceptance of Lamarckism, even into the early to mid 1900s; Gulick 1916, Mayr and Provine 1980), it is perhaps understandable that defense of natural selection was so heated, in response to any apparent exception or perceived replacement to the theory (*e.g.*, Wallace's 1888 disagreement with Gulick). Now, we accept the important role of natural selection in the production of new species (Coyne and Orr 2004) and, generally, the idea that seemingly inconsequential characters might have selective

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value (*e.g.*, intraspecific differences: conspicuous polymorphisms in *Cepaea nemoralis* Linnaeus, 1758 of England, Cain and Sheppard 1950, Provine 1986: 437-456, Brush 2009; interspecific differences: *Mandarina* Pilsbry, 1894 land snails on the Bonin Islands, Japan, Chiba 2004).

However, particularly in the land snail world, we find ample evidence of species that are ecologically similar and exhibit subtle morphological differences. Such species are frequently allopatric, because presumably ecologically similar species cannot remain distinct in the same place at the same time. Clades that have diversified under such conditions can be defined as "nonadaptive radiations," *i.e.*, collections of related ecologically similar species that are allopatric or parapatric replacements of one another (Gittenberger 1991, Rundell and Price 2009). Such radiations include e.g. some groups of salamanders (Wake 2006) and island land snails (Cameron *et al.* 1996, Cook 2008). Note that ecological similarity alone is insufficient for identifying nonadaptive radiation, and the criterion of allopatry (recognized as "isolation" by Gulick) is important.

Gulick, a naturalist, evolutionist, missionary, and malacologist from the Hawaiian Islands, was among the first to describe such patterns of allopatric ecologically similar species, and he used them to assert a potential role for geographical isolation in speciation (Carson 1987). Gulick's contributions to evolutionary theory (A. Gulick 1932, Lesch 1975, Kottler 1976, Reif 1985, Hall 2006a, 2006b) and his life as a missionary and evolutionist (A. Gulick 1932, Amundson 1994) are reviewed elsewhere, as are the general geology and biota of the Hawaiian Islands (Wagner and Funk 1995). Gulick also has been cited for his research on both Polynesian land snails (Cowie 1992, Holland and Hadfield 2004, 2007) and speciation (Mayr 1963a, 1976, 1982, Wright 1978, Price 2008) although his work is still relatively unknown to most biologists. Regarding geology, it should be noted that the utility of the hot spot island chain of the Hawaiian Islands as a potential natural laboratory for the study of evolution and biogeography (*i.e.*, a sequence of islands from youngest to oldest), was not clearly understood in Gulick's time. While early in his career Gulick emphasized that the Hawaiian Islands were distinct from the mainland, each separated by deep channels, and each island contained hundreds of endemic species that had evolved on those islands (Gulick 1853), the theory of hot spot island chain formation was unknown until 1963 (Wilson 1963). Fossils that allowed more accurate dating of islands had only recently been discovered (in the early 1960s; Wilson 1963) and before this, it was assumed that the main Hawaiian Islands were very close in age. It was perhaps extraordinary, then, that Gulick would suggest that Hawaiian snail species might have evolved *in situ* and existed on these isolated islands for "one thousand or ten thousand years" (Gulick 1853: 10).

Given Gulick's careful explication of a pattern that might be common among land snails, I suggest that his research warrants a closer look from a malacological perspective. I also suggest that such insight is necessary for a full understanding of his thinking on geographical isolation. In this paper, I briefly discuss Gulick's early development as a naturalist. I then describe the patterns he saw among the land snails of the Hawaiian Islands. I do not aim to ground-truth each of Gulick's data points, which would require an extensive review of land snail collections in light of the complicated taxonomy of his focal taxa (Cowie *et al.* 1995), some of which is underway (*e.g.*, using Welch's data on *Achatinella mustelina* Mighels, 1845: Holland and Hadfield 2007) although complicated in part by widespread extinction, particularly among the achatinellids and amastrids (Hadfield 1986, Solem 1990, Hadfield *et al.* 1993, Cowie *et al.* 1995, Holland and Hadfield 2007). Instead, I provide a historical framework for understanding Gulick's contributions to our understanding of land snail evolution and the role of geographical isolation. I also suggest that distinctions between diversification patterns in the Hawaiian land snail fauna and other species-rich island land snails illustrate the fortuitous nature of discovery: Gulick's unique position in the Hawaiian Islands and chosen study organisms allowed him a clearer understanding of geography's potential role in speciation than might have been obtained in another study system. Furthermore, by collecting most of what has been written by and about Gulick here, I hope to stimulate future exploration of his work.

Gulick is usually associated with *Achatinella* Swainson, 1828 Hawaiian tree snails (subfamily Achatinellinae, family Achatinellidae) from the island of Oahu (Wright 1978, Carson 1987, Hadfield *et al.* 1993, Cowie *et al.* 1995, Stearns and Stearns 1999, Holland and Hadfield 2004, Hall 2006a), and he frequently referred to other land snails, *e.g.*, small-bodied achatinellids such as *Auriculella* Pfeiffer, 1855, but also the Amastridae, most of which are (or were, before most of the family went extinct; Solem 1990, Cowie *et al.* 1995) ground-dwelling. Gulick placed amastrid species within Achatinellidae. Although it was then clear (Baldwin 1887) as it is now, that Amastridae represent a unique family distinct from the Achatinellidae (Cowie *et al.* 1995; this is also supported by molecular evidence: Holland and Hadfield 2004). Therefore, for clarity, amastrids will be referred to as such throughout, rather than as ground-dwelling members of the Achatinellidae, as they are in Gulick's writings (*e.g.*, Gulick 1872). Hereafter, the ecological categories of "tree" and "ground-dwelling" snails are collectively referred to as "land snails." Tree snails (as defined here) are found on the leaves and bark of live trees, shrubs, and emergent vegetation, whereas ground dwelling snails are found predominantly in or on the leaf litter or rotting logs.

DEVELOPMENT OF A NATURALIST

John Thomas Gulick was born in 1832 on the Hawaiian Island of Kauai (A. Gulick 1932), a few months into the voyage of the *Beagle* (Darwin 1845). His parents were missionaries from the third of twelve companies of missionary ships (the first ship arriving in 1820; Kay 1970) sent to the Hawaiian Islands by the American Board of Commissioners for Foreign Missions to promote Christianity, agriculture, and formal education. Education, which was particularly valued by the missionaries, many of whom had received formal training at northeast American institutions (Kay 1997), spread quickly among Hawaiians, once the missionaries translated Hawaiian language to written form (A. Gulick 1932, Tate 1961). Although it is now clear that missionaries and other westerners had far-reaching negative impacts on the Hawaiian people and environment (Daws 1968), the missionary families' inquisitive minds and adventurous spirit combined with the advantage of settling within the largely unexplored Hawaiian environment, also contributed to important advances in the western discovery and understanding of the biota. They gradually acquired material such as reference books, cabinets, and the occasional microscope to aid in the growing natural history obsession, and sent material abroad for identification, quickly realizing that most of the species surrounding them were unique to the Islands (A. Gulick 1932, Kay 1970, 1997).

Land snail collecting, especially of the multi-colored *Achatinella* tree snails, was a particular passion among Oahu boys and girls alike (Gulick 1853, Kay 1970, 1997). Therefore, Gulick's enrollment in 1842 in the newly founded (1841) Punahou boarding school for missionary children (near what was then considered Honolulu, Island of Oahu), placed him firmly in the midst of "land shell fever" (Baldwin 1887: 2, Kay 1970, 1997), at the age of 10. Subsequently, between visits to Kilauea volcano (Island of Hawaii; Kay 1997), school, religious and agricultural pursuits, Gulick read Darwin's *Voyage of the Beagle* (1845; Carson 1987) and received taxonomic training from Dr. Wesley Newcomb (Gulick Papers 1841-1916, Kottler 1976), a physician who was in residence at Queen's Hospital (Honolulu) from 1850 to 1855 (Abbott and Young 1973, Kay 1997). Newcomb happened to be one of the premier conchologists in North America and maintained correspondence and relationships with leading malacologists such as Gould, Cuming, Sowerby, Ancey, Tryon, and Pfeiffer (Clarke 1960). He traveled broadly and amassed the third most complete shell collection on the continent, which he sold to Cornell University in 1868 for $15,000 (Clarke 1960). These collections included one of the finest sets of Achatinellidae known at the time, which represented years of field work and captive rearing of species in the Hawaiian Islands (Clarke 1960). Gulick's relationship with Newcomb thus proved fortuitous.

In 1853 (at age 21), referring specifically to the influence of the *Voyage of the Beagle* and Dr. Newcomb, Gulick gave "A Lecture on the Distribution of Plants and Animals" before the Punahou Debating Society. Gulick's lecture described principles of adaptation, biogeography, the impact of isolation, particularly on islands, and the striking resemblance of the patterns of endemism among Hawaiian land snails with animals of the Galápagos. This effectively set the stage for what was to become a lifetime of research and promotion of Hawaiian land snails, and the Hawaiian biota in general, as ideal subjects for illustrating evolution and the role of geographical isolation (Gulick 1853, excerpted in A. Gulick 1932: 114-119).

In the years that followed, Gulick studied at Williams College in Massachusetts (graduating in 1859: Parsons 1884), where he continued to present his findings on Hawaiian land snails (A. Gulick 1932: 145) and gained a reputation among his colleagues as a "deep" and serious student and natural historian (A. Gulick 1932: 147). Gulick met Louis Agassiz (famed zoologist, whose work Gulick had read only a few years prior; Kay 1997), who enlisted him to collect in South America, though the trip was cut short due to political unrest in Panama (Hall 2006a). Gulick also read *On the Origin of Species* in the year of its publication (Darwin 1859; A. Gulick 1932, Carson 1987). He studied at Union Theological Seminary and received a Sc.D. from Oberlin (Ohio) and an honorary Ph.D. from Adelbert College (Western Reserve University, now Case Western) in Ohio (Williams College, Class of 1859, Class Letters: Hawaiian Mission Children's Society Library Journal Collection 1819-1900; A. Gulick 1932: 278). Gulick worked as a missionary in Japan and China for about 20 years (during which time he once received severe censure for extreme delay in reaching his post, as a result of evolution (see Kottler 1976: 331-332), personal and snail-related stops abroad; A. Gulick 1932, Carson 1987), interrupted by breaks in the United States, England (where he attended scientific meetings and visited prominent biologists Romanes, Darwin (1872), and others) and elsewhere (A. Gulick 1932: 230). Although Gulick's occupation as a missionary kept him away from the Hawaiian Islands for many years, there is little evidence to suggest that Hawaiian land snail evolution was ever far from his mind (Gulick Papers 1841-1916, Gulick 1885, A. Gulick 1932, Hall 2006a). Although for much of his life, correspondence was Gulick's only lifeline to intellectual peers who shared his passion for evolutionary biology. Nevertheless, Gulick was prolific in writing and speaking on the subject of evolution, and kept sets of snail shells with him for study and discussion. During trans-oceanic travels, he kept many of his collections in New York, and later, on the 1872 trip to England where he met with Darwin, Gulick unsuccessfully attempted to sell his collection to the British Museum (Gulick Papers 1841-1916, A. Gulick 1932, Hall 2006a).

Gulick was married twice and had three children, including a Chinese daughter his first wife (who died in 1875) had saved from a wrecked ship (Parsons 1884). Gulick eventually returned to his beloved Hawaii, participating sporadically in religious, scientific, and educational pursuits as health permitted until his death in 1923 at the age of 91 (A. Gulick 1932). This was an impressive lifespan, given poor health, eyesight, and exhaustion and that frequently plagued him in his early years (A. Gulick 1932: 22, Gulick Papers 1841-1916). The letters, journal entries (Gulick Papers 1841-1916), and publications (including species descriptions: Gulick 1858, Gulick 1873b, Gulick and Smith 1873) left behind now illustrate the development of his theory.

A ROLE FOR GEOGRAPHICAL ISOLATION IN EVOLUTION

The best-known land snail radiation exhibiting little, if any, ecological differences among species is that of the achatinelline tree snails of the Hawaiian Islands, first widely discussed by Gulick (*e.g.*, Gulick 1872, 1873a, 1873b, 1883, 1887, 1889a, 1889b, 1904, 1905). He demonstrated that particularly in the genus *Achatinella* of Oahu, species diversification was promoted by, for example, isolation among geographically distinct valleys, separated by ridges (valley and ridge names illustrated in Fig. 1), and that there was little, if any, difference in the habitat of each species. Gulick also observed differences in individuals' shell characteristics as distance increased from a "source" population.

However, an obvious mechanism by which such prolific diversification of species exhibiting no obvious ecological differences might occur, was initially unclear to Gulick (Gulick 1872). He believed that natural selection could explain adaptive changes within lineages, but it could not explain speciation (Kottler 1976, Reif 1985), particularly when species lived in seemingly identical environments (Gulick 1887, 1889, 1905). Natural selection had strong adherents (*e.g.*, Wallace 1889), and apparent alternatives, such as the one proposed by Gulick that focused on isolation, were sometimes interpreted as claims that natural selection was not the primary mechanism for evolution (Carson 1987), and thus presented a threat to Darwinism itself (Provine 1986: 219-220). Gulick suggested that populations might not only be geographically isolated (*i.e.*, experience "indiscriminate isolation" Gulick 1890e, Reif 1985), but could become "segregated" or experience a reduction in gene flow (Gulick 1905, Reif 1985). "Intensive segregation" (*i.e.*, divergence by natural selection; Gulick 1905, Reif 1985) could occur, but it was ultimately a succession of isolations over long intervals that could lead the way to a new habit in dealing with the environment and formation of divergent species ("cumulative segregation": Gulick 1905; *i.e.*, speciation by divergent evolution, Reif 1985). Unique varieties (*i.e.*, groups within species, showing unique differences in form and color) were considered incipient species (Gulick 1905), which could become species (*i.e.*, strongly pronounced varieties; Gulick 1905). Gulick noted an "inherent tendency to variation" (Gulick 1873a: 499) that we now recognize as genetic variation (Carson 1987). In the absence of a genetic basis for his explanations, Gulick's claim was hard to justify, but attempts to define mutations in the early 1900s eventually supported some of his ideas (Gulick 1905, 1908, Reif 1985). For example, Gulick described a scenario in which a "mutation" of shell coiling direction (chirality) might arise and eventually lead to a new species (Gulick 1905, 1908). We now accept that both isolation and selection operate in the formation of new species (Carson 1987), but Gulick was on the wrong side of the debate when the bulk of his research was published.

One observation that might have lead Gulick to seek an alternative explanation for his observations was that some land snail species occurred on ridges that were connected to one another, rather than just in more geographically isolated valleys. It therefore was unclear to Gulick how such species could differentiate, particularly if they lived on the same plants and had the same predators. Gulick accepted that adaptation could occur (Reif 1985), but he did not find substantive evidence of it in his snails. In Gulick's mind, natural selection could only operate in cases such as the Darwin's finches, where obvious ecological differences and "survival of the fittest" was involved (Gulick 1872, 1873a). Gulick's observations, in contrast, lead him to support nonadaptive explanations (Provine 1986: 216-220).

Although Gulick's speciation mechanism received substantive criticism (*e.g.*, Wallace 1888), it was clear that his novel observations showing the production of many ecologically similar snail species among the ridges and valleys of the Hawaiian Islands might have broader implications for evolutionary theory. By the turn of the century, it was more widely accepted that natural selection alone might be insufficient to explain speciation (Provine 1986: 220). Indeed, Gulick supposed at one point that Wallace did not actually disagree with many of his main propositions (Kottler 1976: 406). Gulick used distribution patterns of achatinellids and amastrids as evidence for a primary role of geographical isolation (Gulick 1887, Reif 1985) in evolution (Gulick 1872, 1873a, 1883, 1889a, 1889b, 1904, 1905). Although his first publications were largely ignored by early Darwinists, Gulick can be partially credited for the eventual acceptance of isolation as an important aspect of speciation (Provine 1986: 220, Carson 1987).

Gulick's data (especially Gulick 1887; Lesch 1975) were identified by George Romanes, a prominent biologist and friend of Darwin, as potential support of Romanes's own

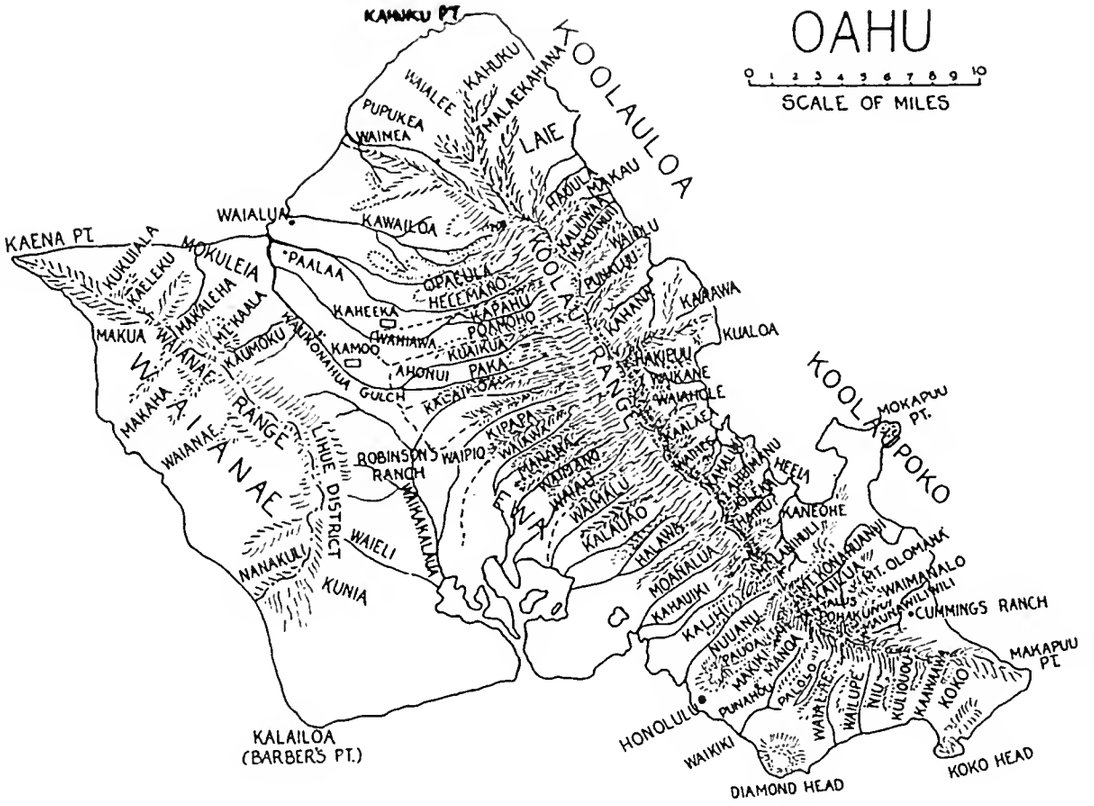

Figure 1. Map of the island of Oahu (Hawaiian Islands). Gulick assembled this map to illustrate his localities in 1873, in response to Wallace's urging in 1872, so that Wallace could include it with Gulick's paper. The process of fruitlessly trying to find a map, and then finally having to assemble it himself using his own memories and checks of Hawaiian oral historic lore, meant that Gulick's paper went before the Linnean Society of London without the map (A. Gulick 1932: 235). Gulick used this map in later years.

ideas on the role of isolation in speciation. These data seemed to provide support for Romanes's theory of physiological selection (Gulick 1906, Lesch 1975, Kottler 1976), which was an unfortunately misleading description for what eventually was known as "reproductive isolation" (Mayr 1982: 565; Kottler 1976; and alluded to in Gulick 1890c, 1908). The close relationship between Gulick and Romanes initially increased the visibility of Gulick's work among prominent biologists (Gulick Papers 1841-1916), but also exposed it to criticism by defenders of natural selection, particularly Wallace (*e.g.*, Wallace 1888; Gulick 1890d,1890f). Romanes, however, finally had empirical data to support his claims, and he eventually blended his own views with Gulick's such that physiological selection was subsumed within the general principle of isolation. He was planning experiments to support these ideas (with Darwin's encouragement) until his premature death in 1894 (Lesch 1975). Romanes's substantial correspondence with Gulick, which was maintained between 1887 and 1894 (Gulick Papers 1841-1916, A. Gulick 1932, Kottler 1976) indicates a richly rewarding scientific relationship that otherwise had been mostly absent from Gulick's adult life, given his isolation as a missionary stationed in the Far East (A. Gulick 1932). However, Gulick's lack of direct assistance in analyzing his own data ultimately might have been a setback in better understanding and promoting his findings.

Despite widespread discussion of ideas on geographical isolation, Gulick's research was not broadly incorporated into evolutionary thought. Among the reasons for this are: Gulick's publication of his largest work (1905) years after Darwin but pre-dating wider acceptance of genetics (Mayr and Provine 1981 Reif 1985) as well as complicated terminology of his papers (Hall 2006a), lack of experimental evidence (Reif 1985), and perhaps his association with the maligned Romanes (Lesch 1975, Kottler 1976). Gulick's ideas were used in support of early Mendelians' theories of saltational/mutational change (perhaps ironically, because Gulick himself was a naturalist, rather than an experimental biologist), as opposed to the majority of naturalists (*e.g.*, Wallace, Hooker, David Starr Jordan, Poulton) whose data supported the idea of gradual evolution by natural selection (Mayr 1980). However, the importance of isolation was gradually gaining acceptance, and geographical isolation's key role in speciation was eventually accepted by naturalists such as Grinnell and D. S. Jordan (Lesch 1975). This provoked a re-examination of past work by, *e.g.*, Moritz Wagner, who was technically the first to describe isolation as critical in species formation (in 1868; Mayr 1963a: 484, Mayr 1982: 562-565) although Gulick came to similar conclusions on isolation independently (Gulick 1890a,1908).

Despite falling out of favor as a result of new studies by "mutationists" in the early 1900s, the important role for geographical isolation in speciation was largely acknowledged by 1942 (Mayr 1982). Coining of the term "isolating mechanisms" (the barriers maintaining reproductive isolation of species; Dobzhansky 1937: 405) was important in this endeavor. This and other work around that time lead to a gradual acceptance of geographical modes of speciation (Mayr 1980: 131). Following the Modern Synthesis, Gulick

was cited by Mayr (1963, 1976, 1980, 1982) and Wright (1978), who recognized the importance of geographical isolation in speciation (*e.g.*, genetic drift; Provine 1986). Gulick's work also laid a foundation for the idea of isolation and random drift leading to nonadaptive differentiation (Provine 1986: 408). Indeed, with the substitution of a few words and phrases, Wright suggested that Gulick's work might describe one of the leading views on how genetic shifts might turn natural selection toward speciation (Carson 1987). Isolation was not emphasized by Darwin, who instead favored pure selectionist explanations for evolution (Carson 1987), but Darwin did discuss the significance of isolation (*e.g.*, Darwin 1872: 81). Darwin's renewed interest in the subject of isolation was indicated in a long meeting with Gulick in 1872 (A. Gulick 1932: 234, Gulick Papers 1841-1916).

WHAT GULICK SAW: PATTERNS OF LAND SNAIL EVOLUTION IN THE HAWAIIAN ISLANDS

Snail diversity and ecology

Gulick focused on two Pacific-endemic land snail families, the Amastridae (326 species) and the Achatinellidae (212 species; Cowie *et al.* 1995), both of which are (or were, prior to the extinction of many species; Solem 1990, Cowie *et al.* 1995) major components of the land snail fauna on the island of Oahu (Fig. 1), where he spent most of his time. These families, which contain some of the largest-bodied, or at least conspicuous, snail species in the Hawaiian Islands, also happen to comprise 70% of described Hawaiian land snail species. (There are 763 nomenclaturally valid species described from the Hawaiian Islands, and almost all of these are endemic, and many are single-island endemics (Cowie *et al.* 1995, Cowie 1996).) The fact that Gulick studied amastrids and achatinellids is not surprising, especially given their beauty and popularity at the time (especially of achatinelline tree snails). Gulick documented about 200 species of amastrids and achatinellids (Gulick 1872, 1883). However, had Gulick lived permanently on the island of Hawaii (the "Big Island") he might have had dramatically different impressions of how evolution proceeded. Here, the Succineidae are (were) most diverse, and exhibit patterns indicative of adaptive radiation, with species' shell shape fitting habitat preferences including waterfalls, tree ferns, leaf litter, and dry scrub areas on the leeward side of Mauna Kea (Rundell *et al.* 2004, Holland and Cowie 2009).

Amastridae

Only a few extant amastrid species remain (Solem 1990, Cowie *et al.* 1995, Holland and Hadfield 2004)—remnants of perhaps the most spectacular, yet least well-known, Pacific land snail radiation. Amastrids are leaf litter or ground dwellers with brownish shell color although much of their ecology remains unknown. They include two subfamilies (genera in parentheses indicate nomenclaturally valid genera *sensu* Cowie *et al.* 1995): Amastrinae (*Amastra* Adams and Adams, 1855, *Carelia* Adams and Adams, 1855, *Laminella* Pfeiffer, 1854, *Planamastra* Pilsbry, 1911, *Tropidoptera* Ancey, 1889) and Leptachatininae (*Armsia* Pilsbry, 1911, *Leptachatina* Gould, 1847, *Pauahia* Cooke, 1911). The genus *Carelia*, restricted to the oldest Hawaiian main islands of Kauai and Niihau, comprises species with the longest shells of any Hawaiian land snail, some species reaching 80 mm (Cowie *et al.* 1995). Gulick's inclusion of amastrids in his papers might be overlooked by non-malacologists because his taxonomy lumped these species together with the achatinellids (*e.g.*, Gulick 1872). Although it appears from his explanations of evolution in the two groups that he did not see a ground-dwelling amastrid species as potentially sister to an achatinellid species (*e.g.*, due to shell chirality differences, Gulick 1872, 1883); certainly this would have significantly changed his conclusions, which were distinct from any notions of adaptive shifts into different habitats.

Nonetheless, Gulick noted potential differences between the ecology of amastrids and achatinellids (Gulick 1872, 1883). He suggested that the ground habitats of amastrids would lead to larger species ranges among these snails, and therefore he categorized them as having a "medium" level of geographical restriction, in contrast to achatinellids, which were highly restricted, and "field" species (possibly invasive species, but this is unclear), which were widespread (Gulick 1872). There has been insufficient research on amastrids to evaluate this hypothesis, but work on other ground-dwelling Pacific land snails suggests that such species can have highly restricted ranges, often more so than tree snails from the same region (Rundell 2010). It is possible that the drab colors of the amastrids, relative to the colorful achatinelline tree snails especially, impacted Gulick's assessment of species, *i.e.*, the more colors, the more "species bins" can be found for those colors. However, Gulick also had the enviable advantage of viewing and collecting amastrids in the wild, and therefore had the opportunity to directly observe interspecific differences. Perhaps Gulick simply possessed less expertise in amastrid taxonomy. Certainly it would be worthwhile to examine his observations in light of significant amastrid museum collections (*e.g.*, Bishop Museum Malacology Collections, Honolulu).

Achatinellidae

Achatinellids are some of the best-known land snails in the world. Most museums have at least one drawer full of these colorful banded or jewel-like tree snails (*i.e.*,

achatinellines), yet these are but a subset of the diversity contained within the group. There are five subfamilies: Achatinellinae (including *Achatinella*, *Newcombia* Pfeiffer, 1854, *Partulina* Pfeiffer, 1854, and *Perdicella* Pease, 1870, but subgenera such as *Bulimella* Pfeiffer, 1854 are also discussed by Gulick (1872, 1889b)); these species reach the largest body sizes of any Pacific achatinellids), Auriculellinae (*Auriculella*, *Gulickia* Cooke, 1915), Pacificellinae (*Lamellidea* Pilsbry, 1910, *Pacificella* Odhner, 1922), Tornatellinae (*Philopoa* Cooke and Kondo, 1961, *Tornatellaria* Pilsbry, 1910, *Tornatellides* Pilsbry, 1910), and Tornatellininae (*Elasmias* Pilsbry, 1910). The number of synonymies in this family (*e.g.*, summarized by Cowie *et al.* 1995) suggests that the family Achatinellidae has been subjected to over-splitting; however, some experts have suggested that species richness in Hawaiian land snails, even achatinellids, has been underestimated (Solem 1990).

The observations on which Gulick based his theory of evolution through nonadaptive means and isolation, and then spent most of his life defending, were largely of the *Achatinella* of Oahu, which were some of the most brilliantly and variably colored achatinellids (*e.g.*, Hall 2006a). Gulick also frequently referred to "*Helicteres*" (*sensu* Gulick 1873b), which was a subgenus of achatinellines (*e.g.*, as is *Bulimella*). "*Helices*" are also mentioned, and depending on the context, are usually simply other species of amastrids or achatinellids (Gulick 1889b) although "*Helix*" likely refers to planispiral snails such as helicarionids (*e.g.*, Gulick 1872).

Among the most critical facts regarding the natural history of most achatinellines, was the snails' fidelity to trees, which they were unlikely to leave for their entire life history, as well as the preference of achatinellines for shady groves within valleys, that were separated from other shady groves by drier areas (Gulick 1905: 220). For some species, per-tree population densities were estimated to be 500 individual adult snails per tree or 2000 adults and juveniles per tree (*i.e.*, *Partulina confusa* Sykes, 1900: Hadfield 1986). An individual snail's fidelity to the same tree, sometimes throughout much of an individual snail's life (Solem 1990, Hadfield *et al.* 1993), could have important consequences for restriction of gene flow. This extremely low-dispersal lifestyle might dramatically impact speciation in this group, contributing to subdivision of populations that could remain intact for long periods of time, perhaps slowly accumulating mutations (*e.g.*, Price 2008) or, as Gulick suggested at the time (though little evidence existed for the his idea) "spontaneous variations" could arise (Gulick 1872), *e.g.*, in a new direction of shell chirality in two mating individuals from a single tree (Gulick 1905: 68-70, 1908). However, Gulick did not expect that this was the predominant means of species formation (Gulick 1905: 70).

It could be argued that the ground-dwelling amastrids would have less restricted species ranges, because individuals might be free to roam the seemingly endless, interconnected ground-scape (as Gulick inferred; Gulick 1872), in contrast to the individual trees inhabited by achatinelline tree snails. I think this outlook neglects the inherent patchiness of leaf litter and logs, and perhaps the preferences for different amastrid species to prefer some dead leaves to others; however, the ecology of most amastrids will never be well known, because the majority of species are now extinct. Thus, it is possible that both amastrids and achatinellids had equally restricted ranges, which, although suggested by the large species numbers in both families, did not seem immediately clear to Gulick. However, he was quite interested in exploring this idea among Kauai *Carelia* (Gulick 1872), which would have been isolated between steep ridges in some areas, such as the Na Pali coast (Solem 1990).

Island geology, isolation, and similar environments

Beginning in his 1872 paper and re-stated in other papers (Gulick 1873a, 1883, 1905), Gulick established the following patterns illustrating the impact of geographical isolation (at different scales) and island size on speciation. Islands tend to have unique faunas (*e.g.*, land snails of Cuba; Gulick 1872) and the achatinelline tree snails are completely unique to the Hawaiian Islands. Within this island group there are different achatinelline genera and these are distributed throughout the valleys of the Hawaiian Islands. Species on Kauai are more distinct from species of Oahu and Maui, for example, than those islands' species are from each other and might have dispersed, for example, by being carried by birds (Gulick 1873a, 1910). There are no Recent *Achatinella sensu stricto* Cowie *et al.* 1995 on Kauai; there is a putative subfossil achatinelline, *Newcombia* (Gage 1996) although data suggest it represents a back-colonization from younger islands (Holland and Hadfield 2004). However, there are Amastridae (*i.e.*, *Carelia* (Cowie *et al.* 1995) unique to Kauai and Niihau), which might indeed be considered quite distinct from amastrids of other main Hawaiian Islands.

Gulick also remarked that substantial speciation has taken place in a very small area, particularly among achatinellids and amastrids, so that most species are single-island endemics and within Oahu most species are restricted to one mountain range, the Koolaus, an area 40 miles (64.4 km) long by 5 to 6 miles (8.0 to 9.7 km) wide (Gulick 1872), each species having a range of only 1 to 5 miles (1.6 to 8.0 km; Gulick 1872). Both the ideas of isolation of island archipelagos and isolation within those archipelagos have been cited as potentially important drivers of speciation, but in the mid to late 1800s, these ideas were still relatively new. What made Gulick's perspective unique was its emphasis on the impact of subdivision of isolated areas (*e.g.*, within mountain ranges such as the Koolau Mountains of Oahu) on species diversification within a relatively small area. Among the achatinellids

and amastrids, but *Achatinella* in particular, sometimes unique species were found in neighboring valleys (Gulick 1872).

The orientation of the main mountain range that Gulick studied and that was home to many species of *Achatinella*, the Koolau Mountains of northwestern Oahu (Fig. 1), turned out to be instrumental in showing a clear role for geographical isolation of populations and speciation. These mountains have an elongate "spine" with parallel valleys arranged along their length (A. Gulick 1932). In contrast, Gulick noted that varieties were less distinct on Maui, and it was therefore more difficult to connect species using the gradations in color or pattern of different varieties. He attributed this problem to the concentric arrangement of valleys on Maui (Gulick 1872). This geological pattern might have presented a similar challenge for studies of *Partula* Férussac, 1821 tree snails in French Polynesia (Crampton 1916, 1932); although the arrangement of some valleys, *e.g.*, on Moorea, resembles that of valleys in the Koolaus, and there is greater potential for contact at either end of the mountain range (Clarke and Murray 1969, Murray and Clarke 1980). Notably, Crampton (1932: 188; Provine 1986: 437) found support for nonadaptive differences in size, shape, and color of *Partula* snails, whereas later study demonstrated adaptive habitat partitioning among coexisting *Partula* species (Murray and Clarke 1980, Cowie 1992). Ecological and biogeographical distinctions between the Hawaiian achatinellines and French Polynesian partulid tree snails that apparently resulted in such dissimilar radiation patterns still warrant further investigation (*e.g.*, as was suggested by Cain and Sheppard 1950; Provine 1986: 442-443).

The most controversial part of Gulick's hypothesis on diversification through "nonadaptive" means (Hall 2006a) was his insistence that habitats on either side of a ridge, for example, were identical (Gulick 1872, 1889a). Gulick gave careful descriptions of snail habitats that supported this view (Gulick 1872, 1889a). He could find no clear adaptations to specific environmental conditions or enemies on either side, despite Wallace's insistence that all environments are different (Wallace 1889: 149). Gulick did note that some species preferred certain trees (*e.g.*, *Bulimella*'s preference for kukui trees (*Aleurites moluccana*; Gulick 1872)), and he acknowledged the role of selection for certain species, yet his observations of most other snail species lead him to nonadaptive explanations (Gulick 1885, 1897, 1904, Kottler 1976: 330, Hall 2006a). Plant species were sufficiently widespread on the islands that there seemed to be no difference from valley to valley. It is difficult today to clearly understand potential habitat preferences of snail species because a large proportion of the indigenous Hawaiian forest has been destroyed. Thus, it is not evident whether current plant preferences (which indicate the setting in which snails can glean fungus from leaves or bark) accurately represent the breadth of species on which the snails once lived (Gulick 1905). Modern populations of *Achatinella mustelina* are known to occur on several indigenous plant species (Hadfield *et al.* 1993), and several *Achatinella* species can survive on the common indigenous forest tree *Metrosideros polymorpha* (ohia-lehua) supplemented by cultured fungus (Rundell 2000, pers. obs.; Stearns and Stearns 1999: 33). It is possible different plant species might have historically harbored different fungal species on their leaves and bark, which the snails consumed, but this is unknown. It is also possible that this fungal diversity might have declined, with the extinction or reduction of indigenous plants.

It was unclear to Gulick why species would not "pass over their narrow bounds and become mingled" (Gulick 1872: 223). After all, species occurred not just in the valleys, but on the ridges; indeed ridgetops are all that remain of some species' distributions (*e.g.*, Hadfield *et al.* 1993). Slow dispersal and fidelity to the tree habitat, as noted by Gulick (indeed, fidelity to individual trees might be common, as in *Achatinella mustelina*: Hadfield *et al.* 1993), in combination with gradual acquisition of mutations might help to account for this (Gulick 1905, 1908), but obviously Gulick posed a very interesting question. It might have been the observation of ridgetop snail species and varieties that lead him to believe that geographical isolation alone was insufficient for speciation to occur (*e.g.*, Gulick 1872, 1873a: 500, 1905: 221). Gulick also explored the possibility that ridge-dwelling species might have a higher dispersal capacity than valley-dwelling species (Gulick 1905: 221). He later indicated that these separate populations would "develop different types of variation" and would eventually be "liable to subject themselves to different forms of selection" (Gulick 1914: 63).

Populations, varieties, and change with distance

The fact that subtle gradations of shell phenotypes could be found linking species, and as distance increased, the difference in phenotypes also increased, also seemed to support Gulick's notion that the process of speciation was largely driven by degrees of geographic isolation. Such data were acquired by careful collection of the many different shell phenotypes Gulick observed in each land snail population (A. Gulick 1932). Given the current extinction crises, particularly among Pacific island land snails (Solem 1990, Gould 1991, Cowie 1996), but especially among achatinelline species with low reproductive rates (Hadfield *et al.* 1993), the collection of the great number of individuals that this method required now seems outrageous (*i.e.*, Gulick collected and procured at least 44,500 shells in three years; Hadfield 1986, Stearns and Stearns 1999). It is difficult to imagine that Gulick and his contemporaries did not directly contribute to the decline of some achatinelline snail species since literally thousands of slow-to-reproduce adult snails were commonly collected or procured from indigenous Hawaiian collectors in a single day

(Hadfield 1986). Although at some localities, there were signs that species might have been already in decline (*e.g.*, in 1853: A. Gulick 1932: 123), in some cases as a result of overgrazing (Gulick 1873a: 504, Baldwin 1887), particularly in the 30 years following the release of cattle by Captain George Vancouver in 1804 (Kay 1997). The tragedy of snail "deserts" where once there were literally tens of thousands of individuals (Hadfield 1986, Stearns and Stearns 1999) cannot be disputed.

However, when viewed within a historical context, Gulick's desire to collect each potential phenotype within a population reflected progressive, non-typological thinking (*e.g.*, Reif 1985). Certainly some taxonomic divisions below the species level might have no genetic significance, when analyzed using modern techniques (*e.g.*, Waianae Mountains, Oahu *Achatinella mustelina* subspecies named by Welch (1938); Holland and Hadfield 2007), but there is likely some information contained within the many subspecies, varieties, and variations identified by Gulick and others. For example, in a preliminary study, Pelep and Hadfield (2010) found that some genetic differences corresponded with differences in shell shape. Gulick identified *ca.* 800 to 900 varieties of Oahu snails from his focal taxa (Gulick 1872), which he supposed to be "segregated" from other varieties (Gulick 1905: 222).

Regardless of the ultimate status of these different phenotypes, Gulick seemed to intend them as evolutionary works in progress and raw material for understanding variation, rather than immovable types (Gulick 1872, 1883). Some variations were well-known to him to be varieties of the same species, but others were not (Gulick 1889b: 348). Gulick also knew that there was not a new species in every valley—some species spanned multiple valleys (Gulick 1872, 1883). But within these wide-ranged species there were varieties (*i.e.*, groups within species, showing unique differences in form and color) and these showed population diversity (Gulick 1889a). Varieties would grade into one another over distances across the range of a species (Gulick 1872, 1889a). Collection of a "series" within populations and species (Gulick 1872; *i.e.*, collecting all potential varieties or color morphs of a species, for which achatinellines possessed many: Gulick 1889b) was not uncommon among systematists, even before Darwin (1859), because these early biologists recognized that individuals of a species are not identical (Mayr 1980). Gulick suggested that understanding the patterns of minute gradations (*i.e.*, differences in form and color) would help him understand the evolution of species in the context of the geography of an area and distance from the "home of the type" (*i.e.*, a typical form of the species selected by Gulick: 1889b: 347; Gulick 1858, 1872, 1889a; also see Mayr 1980: 130). The different banding patterns and colors among the achatinellines was of great interest, but Gulick could not understand how these differences correlated with plant preference (Gulick 1872). In other land snails (*e.g.*, *Cepaea*) such differences have been shown to have adaptive significance (Cain and Sheppard 1950, Provine 1986); however, no adaptive significance has been found for color or banding variation in achatinelline tree snails.

Wallace did find exception with some of Gulick's explanation of diversity within populations. He argued that some of the forms described by Gulick (*e.g.*, his explanation of "varieties"; Gulick 1889b) should only be considered "variations" which could not be counted as "taxonomically significant varieties" that arose when natural selection had acted upon them. Wallace observed that "variations" would spring up without dependence on the environment (Gulick 1890b, A. Gulick 1932: 462). In fact, this criticism seemed to reiterate Gulick's observations (Kottler 1976), and Gulick emphasized that Wallace had not demonstrated the environmental differences of which he spoke (Kottler 1976: 330). Criticism notwithstanding, Gulick was also concerned with careful documentation of each locality from which he gathered or purchased his populations of snails (Gulick 1872, A. Gulick 1932, Gulick Papers 1841-1916). He stated that "Each valley, with its area two to three miles in length, and but one or two miles in width, needs to be separately explored, and all the shells labeled with the name of the valley" (Gulick 1872: 224). This was a novel approach in an age when "Sandwich Islands" was considered sufficient data (Cooke 1941) although some of the localities in Gulick's descriptions (*e.g.*, Gulick 1873b) report "Sandwich Islands" for the locality. It is possible that Gulick did not recognize the importance of recording exact localities in his early field work and shell procurement activities with his Hawaiian collectors. Given the lack of detailed locality information in many of Newcomb's (Gulick's first mentor) collections (Clarke 1960), it is doubtful this was a lesson Gulick learned early on, before he was conversant in evolutionary theory. It is perhaps worth remembering that Darwin himself was not always careful to label *e.g.*, finch species, even according to their island of collection (Carson 1987).

However, Gulick's data were sufficient to demonstrate the effects of isolation on evolution. He noted that in one genus from one mountain range, species were connected by varieties with minute gradation in form (*i.e.*, shell shape) and color (Gulick 1889a), whereas species of the same genus on different islands were not so completely connected by intermediate forms. Gulick observed that the degree of difference between several species in the same group was in proportion to the species' separation in space (Gulick 1872, 1883, 1905).

CONCLUSIONS

Comparisons with other island land snail studies

Modern studies have found that geographical isolation likely plays an important role in island land snail evolution

(Solem 1984), for example among land snails of the Atlantic Madeiran archipelago (Cook 1996, Cook 2008), and Lord Howe Island and Rapa (Solem 1984). Ecological differences have been suggested to place a strong role in completion of the speciation process (*e.g.*, different hill habitats of Cook 1996), and it has also been suggested that neutral processes might account for high species richness at any one site (Cook 2008). Solem (1984) refers to the latter as a "mosaic assemblage" in which species are added to an assemblage with little or no competitive exclusion. Here, scattered clusters of different species can be associated with specialization on particular plant communities. The two ideas seem to run counter to one another: at the beginning of the process not only isolation, but adaptation to unique habitats was required for speciation, but individual local land snail communities comprised "weakly interacting" species (Chiba 2007).

Gulick demonstrated that species diverged in nearly identical environments on either side of a barrier, and very few adaptations to local environments could be found. Wallace's criticism that we do not know enough about these snail species to assume that ecological differences do not exist (*e.g.*, Wallace 1889: 148), remains largely unanswered, except by Gulick himself (*e.g.*, Gulick 1905), and modern observations that several species can survive on one indigenous tree species, *Metrosideros polymorpha* (ohia-lehua; Stearns and Stearns 1999, Rundell 2000, pers. obs.), and some species can survive on more than one indigenous plant species (Hadfield *et al.* 1993).

The resulting communities of more distantly related species in the valleys and on ridges were also species-rich, but Gulick was less concerned with community-level patterns (but see *e.g.*, Gulick 1889a), which have lead to the more recent ideas of "nonadaptive" influences on land snail diversification, as in studies mentioned above. Furthermore, there are differences in habitat types and the distribution of those habitats, between the Hawaiian Islands, and for example, the Madeiran Islands. Whereas Gulick noted patches of landscape (*e.g.*, "meadows") distinct from the rainforest, generally the amastrids and achatinellines were not present there, and so these patches only served to further isolate his species (Gulick 1872). The rainforest where most of Gulick's species lived was relatively uniform. In contrast, in the Madeiran Islands, the variety of landscapes augments species diversity within a clade and provide unique microhabitats to which species adapt. Within each habitat, convergence in shell shape may occur (*e.g.*, as with Bonin Islands *Mandarina*; Chiba 2004).

These brief examples demonstrate the variety of ecological and evolutionary processes that may contribute to land snail diversification. Investigation of the intersection between radiations of species (nonadaptive or adaptive) and subsequent community assembly involving those species, is clearly important for a better understanding of land snail evolution on islands. In some sense, Gulick's study system choice was fortuitous in providing the most straightforward example of geographical isolation and the effects of distance on species differentiation.

Future directions

A few great malacologists followed, and were coincident with Gulick in documenting Hawaiian land snails, including Cooke, Welch, and Kondo (Cooke 1941, Cooke and Kondo 1960; reviewed by Solem 1990), and it is on their collections that we must now base a large part of our research on diversification in these spectacular snails. Unfortunately, these pioneers of Hawaiian malacology did not produce enough students or intense interest in the snail fauna when these species were still extant, and so it may be too late to ask some of the important evolutionary and ecological questions of these snails (Solem 1990). Certainly, malacology has rarely been part of the "bandwagon effect" lamented by Simpson and Mayr, where most of the attention, resources, and bright students flock to fields that are technologically or conceptually advancing at a rapid rate, promising fame and fortune (Mayr 1963b: 1, Simpson 1964: 113-114, Beatty 1994: 348).

But magnificent museum collections remain, and combined with populations surviving in the wild and in captivity, we can learn a great deal (Hadfield *et al.* 1993, Holland and Hadfield 2004, 2007). Focus on the less charismatic, non-achatinelline achatinellid families (although many species are also likely extinct; Solem 1990) might also reveal fascinating patterns based on molecular data, that could be compared with Gulick's ideas (*e.g.*, R. H. Cowie, unpubl. data on tornatellinine snails). Collections-based studies of shell shape and internal morphology, particularly of the amastrids, could also provide important insights. In this sense, amastrid collections are the best-preserved "fossils" in the world; indeed, there are also fossil and sub-fossil amastrids that could be included in analyses of Recent amastrid taxa.

Perhaps we could approach museum collections with the same spirit of Gulick, in the largely unexplored (by western eyes, at least) Hawaiian Islands. "Here we are, by fortune or by providence, placed in the midst of an unexplored field, which promises to the student of nature the richest rewards: and what boy, what girl—we may rejoice that we are boys and girls when we see what there is yet for us to learn—but who is here amongst us that cannot do something to advance the cause of science, if he will only commence now..." (Gulick 1853: 10).

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The opposite of speciation: Genetic relationships among the populations of *Pleurocera* (Gastropoda: Pleuroceridae) in central Georgia*

Robert T. Dillon, Jr. and John D. Robinson

Department of Biology, College of Charleston, Charleston, South Carolina 29424, U.S.A.
Correspondence, R. T. Dillon: dillonr@cofc.edu

Abstract: The ranges of *Pleurocera* (formerly *Goniobasis* or *Elimia*) *catenaria* (Say, 1822) and *P. proxima* (Say, 1825) extend from Virginia south through the Carolinas into the piedmont and upper coastal plain of Georgia, where they intersect with populations of *P. floridensis* (Reeve, 1860). In contrast to the situation in surrounding states, however, Georgia populations of *P. catenaria* have been taxonomically subdivided and re-described under at least ten specific nomina, over a complex monographic history extending 150 years. To see if this increased nomenclatural diversity might signal higher levels of population divergence, we compared gene frequencies at 11 polymorphic allozyme-encoding loci among eight populations of *P. catenaria* and three populations of *P. floridensis* sampled from central Georgia region to three populations of *P. proxima*, which have never been taxonomically subdivided. Genetic variation was moderate within our 14 populations and high among them, as has been reported in many prior surveys of pleurocerid allozyme divergence conducted elsewhere. The pairwise genetic distances demonstrated among our populations of *P. catenaria* from Georgia were lower than those observed among control *P. proxima* populations, or among a comparable sample of *P. catenaria* populations from the Carolinas previously published. Central Georgia does not appear to be a region of pleurocerid endemicity, but rather of faunal suturing, the proliferation of specific nomina attributable to qualitatively higher levels of shell morphological variation, possibly ecophenotypic in origin. Junior synonyms of *P. catenaria* include *albanyensis, boykiniana, caelatura, darwini, mutabilis, postelli, suturalis* and *viennaensis*. Junior synonyms of *P. floridensis* include *inclinans, induta, exul*, and *nymphaea*. The Goodrich nomen *timidus* is retained as a subspecies of *P. floridensis* (new combination). We suggest that faunas demonstrating great evolutionary stasis, such as the pleurocerid populations of central Georgia, might profitably serve as the next "laboratories of speciation."

Key words: freshwater snails, divergence, allozyme electrophoresis, *Goniobasis*, *Elimia*

The evolution of North American pleurocerid gastropods has been a subject of research interest for many years (*e.g.*, Adams 1915, Goodrich 1935, Chambers 1978, Dillon 1989, 1991, Holznagel and Lydeard 2000, Dillon and Robinson 2009). Their wide distribution and great abundance in rivers and streams throughout the continent, together with their striking genetic and morphological diversity, have made pleurocerid populations ideal models for the study of gene flow (Dillon 1988a), natural selection (Dillon 1984, 1988b), hybridization (Bianchi *et al.* 1994), divergence (Goodrich 1922, 1936, Lydeard *et al.* 1997, Dillon and Lydeard 1998), speciation (Chambers 1980, 1982, Dillon and Ahlstedt 1997) and phenotypic plasticity (Dillon, in press). But the same attributes that today render pleurocerid populations so attractive as evolutionary models also led nineteenth-century biologists to describe hundreds of pleurocerid species throughout North America (Tryon 1873, Graf 2001), yielding great taxonomic confusion.

Recently we have reviewed diverse lines of genetic, biogeographic, and ecological evidence suggesting that pleurocerid populations inhabiting the piedmont and Blue Ridge provinces of the southern Appalachians may be extremely old—perhaps dating to the Appalachian orogeny 300 mybp (Dillon and Robinson 2009). We have reported double-digit mtDNA sequence variation both within and among three sets of conspecific pleurocerid populations, correlated neither with simple overland distance, nor with continental drainage patterns as the rivers currently flow.

The genus-level taxonomy we employed in our 2009 survey has more recently been revised, the pleurocerid genera *Goniobasis* and *Elimia* being subsumed under *Pleurocera* by Dillon (in press). But the species-level taxonomy of most of the 13 populations we surveyed was uncontroversial. *Pleurocera* (formerly *Goniobasis* or *Elimia*) *proxima* (Say, 1825) is easily recognized throughout its entire five-state range (Dillon 1984), and *Pleurocera* (formerly *Goniobasis* or *Elimia*) *catenaria* (Say, 1822) is well-characterized and taxonomically stable through Virginia and the Carolinas (Dillon and Reed 2002). In Georgia, however, populations morphologically indistinguishable from *P. catenaria* have been referred to at least ten specific nomina in the modern

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literature, including *albanyensis* (Lea, 1864), *darwini* (Mihalcik and Thompson, 2002), *inutabilis* (Lea, 1862), *postelli* (Lea, 1858), *suturalis* (Haldeman, 1840), *tinidus* (Goodrich, 1942), and *viennaensis* (Lea, 1862). The two populations of *P. catenaria* we sampled in 2009 from the Georgia piedmont have, in recent publications, been identified as *Goniobasis* (or *Elimia*) *boykiniana* (Lea, 1840), *caelatura* (Conrad, 1849), or *lecontiana* (Lea, 1841). Further taxonomic complication is added by populations of *Pleurocera* (formerly *Goniobasis* or *Elimia*) *floridensis* (Reeve, 1860), a biologically distinct species ranging into Georgia from the south, sometimes nearly indistinguishable from *P. catenaria* (Chambers 1978). The purpose of the present research is to determine whether the great nominal diversity that has been attributed to the *Pleurocera* populations of central Georgia reflects *bona fide* genetic divergence, or whether such secondary factors as ecophenotypic plasticity of shell or taxonomic artifact may be responsible.

Through extensive application over 30 years, the technique of allozyme electrophoresis has proven to be a valuable tool both for quantifying genetic diversity, as well as for resolving the specific status of problematic pleurocerid populations. Levels of divergence are best understood in *Pleurocera proxima*, from which dozens of populations have been studied in four states, with calibration against breeding data (Dillon 1986, 1988b) and mitochondrial sequence divergence (Dillon and Frankis 2004). In the present survey we compare levels of divergence at allozyme-encoding loci among populations of *P. proxima* and *P. catenaria* from the Carolinas, whose conspecific status is uncontroversial, to the levels of divergence in a larger sample of Georgia *Pleurocera* populations variously identified under nine specific nomina, whose true status as biological species is far from clear.

MATERIALS AND METHODS

We sampled 11 populations of *Pleurocera* from ten rivers and streams in central Georgia, selected to represent the taxonomic diversity, geographic range, and morphological variability of the genus in the region. We also sampled three control populations from adjacent states: *P. proxima* and *P. catenaria* from South Carolina, and *P. floridensis* from Florida. The locations of these 14 populations are shown (Fig. 1), and detailed locality data are given in Appendix 1, along with sample sizes and notes regarding the specific identifications that have been accorded these populations by previous investigators. Sample sizes were in almost all cases greater than 30, but smaller for the populations previously sampled by Dillon and Reed (2002): **Prx1** ($N = 5$), **Catn** ($N = 5$), and **Mut** ($N = 22$). These three smaller data sets were combined with our 2002 data ($N = 29$, 38, and 40, respectively) before analysis. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia.

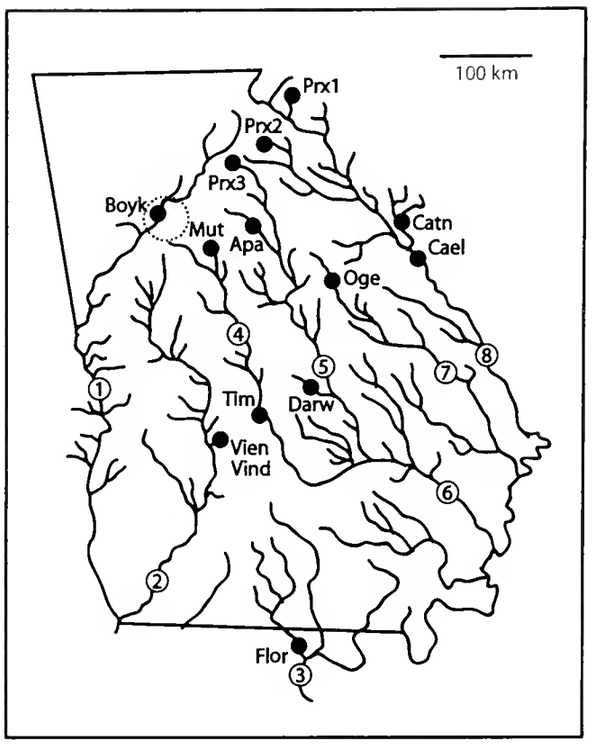


Figure 1. Map showing sample sites in Georgia and adjacent states (see Appendix 1 for a key to site names and detailed locality data). Major rivers are (1) Chattahoochee, (2) Flint, (3) Withlacoochee/Suwanee, (4) Ocmulgee, (5) Oconee, (6) Altamaha, (7) Ogeechee, and (8) Savannah. The Atlanta metropolitan area is indicated with a dashed circle.

Animals were returned alive to the laboratory, where they were cracked and frozen in tris tissue buffer until electrophoretic analysis. Our techniques and apparatus for horizontal starch gel electrophoretic resolution of allozyme variation in homogenates of molluscan tissues were detailed by Dillon (1992), along with recipes for all buffers and stains employed here. The Tris Cit 6 buffer (buffer XIII of Shaw and Prasad 1970) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), glucose-phosphate isomerase (GPI), and isocitrate dehydrogenase (two loci, the cathodal IDHF and the anodal IDHS). A Poulik (1957) discontinuous buffer system was employed for phosphoglucomutase (PGM—the strong, fast locus only), sorbitol dehydrogenase (SDH), and octopine dehydrogenase (OPDH). The TEB8 buffer system (buffer III of Shaw and Prasad 1970) was used to analyze xanthine dehydrogenase (XDH), mannose phosphate isomerase (MPI), and PGM, the single locus resolved being identical with the strong fast locus resolved using the Poulik buffer. A TEB9.1 buffer (Dillon and Davis 1980) was used for octanol dehydrogenase (OLDH), esterases (EST1—the strong, slow locus only), and XDH (a second time).

Mendelian inheritance of allozyme phenotype has been confirmed for GPI, OPDH, and EST1 by Dillon (1986) and for 6PGD by Chambers (1980). Putative allelic designations

for each zone of allozyme activity were assigned using the system of Dillon and Reed (2002), setting the shared populations as standards. For example, since Dillon and Reed designated the OPDH allele for which population **Prx1** was fixed as "106," the new OPDH allele discovered in population **Tim**, with a gene product migrating 4 mm faster than that of OPDH106 in our standard conditions, was named "OPDH110."

Gene frequencies and mean direct-count heterozygosities (the unbiased estimate of Nei 1978) were calculated using Biosys version 1.7 (Swofford and Selander 1981). Because large numbers of alleles were resolved at some loci, our sample sizes dictated that genotypes be pooled into three classes before testing for Hardy-Weinberg equilibrium: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes. Yates-corrected chi-square statistics were then employed for this purpose. We calculated matrices of Nei's (1978) unbiased genetic identities and distances, as well as Cavalli-Sforza and Edwards (1967) chord distance. As distances of the latter type are Pythagorean in Euclidean space, they were used as the basis for the construction of a neighbor-joining tree using Phylip v3.65 program NEIGHBOR (Felsenstein 2004).

An $N \times N$ symmetric matrix contains only N - 1 statistically independent entries, carefully chosen. So as a measure of genetic divergence within the Georgia populations of *Pleurocera catenaria*, we used their symmetric matrix of Nei's unbiased genetic distances to construct a minimum spanning network with the method of Prim (1957). We also extracted N - 1 independent segments from the symmetric matrices of genetic distances among the 7 populations of *P. catenaria* sampled from the Carolinas by Dillon and Reed (2002), and among the 6 populations of *P. proxima* sampled in the two studies combined (three unique from 2002, two unique from the present study, one shared). Then we used Mann-Whitney U statistics to test the one-tailed hypothesis that the genetic divergence among Georgia populations of *P. catenaria* might be greater than either the Carolina populations of *P. catenaria*, or the *P. proxima* populations sampled from across the three states combined.

RESULTS

Typical shells sampled from our control populations of *Pleurocera proxima*, *P. catenaria*, and *P. floridensis* have been figured previously (Chambers 1980, Dillon and Reed 2002). Our fresh samples from central Georgia displayed striking shell morphological variation, especially with regard to strength of shell sculpture (Fig. 2). Costation and carination demonstrated great variability both within and among populations, and did not suggest to us any taxonomic significance. We were impressed, however, by the systematic slenderness

Figure 2. Example shells from populations **Vien**, **Darw**, **Vind**, and **Tim** (left to right). For others see Dillon and Reed (2002) and Chambers (1980, 1990).

of the shells borne by snails of populations **Vind** and **Tim**. Although the shells we sampled from most central Georgia populations of *Pleurocera* were characterized by relatively long body whorls, as demonstrated by the two specimens at the left of Fig. 2, populations **Vind** and **Tim** displayed narrower shells with body whorls shorter in proportion to total shell length.

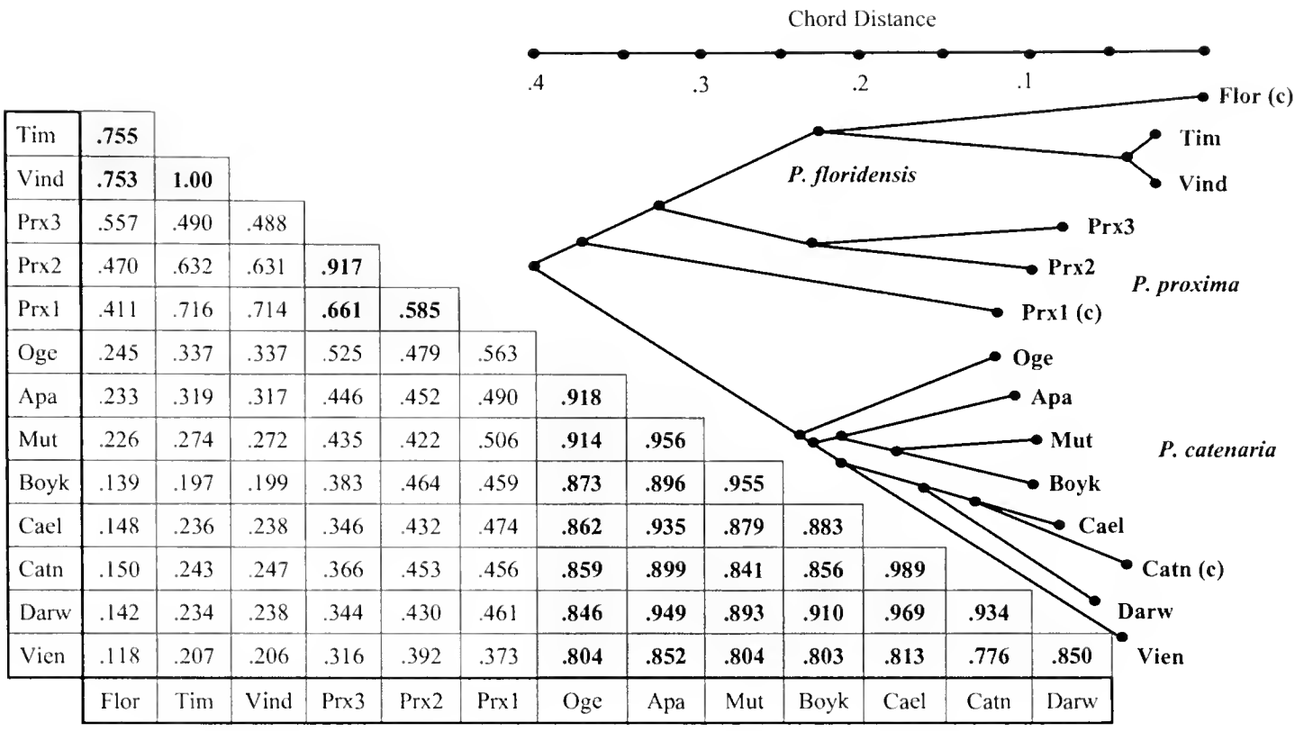
Putative gene frequencies at the 11 allozyme-encoding loci we examined are given in Table 1, together with mean direct-count heterozygosities. Intrapopulation genetic variation was moderate in our sample of 14 *Pleurocera* populations from three states, and interpopulation divergence high, as has often been reported in surveys of pleurocerid population genetics (Dillon and Davis 1980, Dillon 1984). Of the $11 \times 14 = 154$ loci examined, 37 were polymorphic by the 95% criterion. The lowest value of P returned by goodness-of-fit tests to Hardy-Weinberg expectation among any of these was $P = 0.015$ (PGM in population **Cael**, $\chi^2 = 5.89$), which is not significant with Bonferroni correction ($0.05/37 = 0.0014$). Thus our data contained no evidence that any assumption of Hardy-Weinberg equilibrium has been violated within populations.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown (Fig. 3), together with the results of our neighbor-joining analysis based on Cavalli-Sforza and Edwards chord distances. Seven Georgia populations clustered in a group together with our control population of *Pleurocera catenaria* from South Carolina (**Catn**), and two Georgia populations clustered with our control *P. floridensis* (**Flor**). Our three populations of *P. proxima* were depicted as central in the network, more loosely clustered than either *P. catenaria* or *P. floridensis*.

Values of Nei's (1978) unbiased genetic distances are compared from the survey of Dillon and Reed (2002) in North Carolina and South Carolina to values obtained in the present study (Fig. 4). For this analysis population **Catn** was grouped with the 2002 Carolina data, and population **Mut**

Table 1. Gene frequencies and average (direct count) heterozygosity over eleven polymorphic enzyme loci in 14 populations of *Pleurocera* from Georgia and surrounding states.

	P. floridensis			*P. proxima*			*P. catenaria*							
allele	Flor	Tim	Vind	Prx1	Prx2	Prx3	Apa	Boyk	Cael	Catn	Darw	Mut	Oge	Vien
GPI														
100	.000	.000	.000	.000	.000	.000	1.000	1.000	.500	.202	1.000	1.000	.567	1.000
102	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.000	.000	.000	.000	.000	.000	.500	.750	.000	.000	.433	.000
110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.048	.000	.000	.000	.000
MPI														
95	.097	1.000	1.000	.026	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
98	.903	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
100	.000	.000	.000	.974	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6PGD														
100	.000	1.000	1.000	.897	1.000	.967	.973	.597	.935	1.000	1.000	.515	1.000	1.000
103	.258	.000	.000	.000	.000	.033	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.000	.103	.000	.000	.027	.403	.065	.000	.000	.394	.000	.000
107	.742	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.091	.000	.000
EST1														
100	1.000	1.000	1.000	.218	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
103	.000	.000	.000	.769	.000	.000	.886	.532	1.000	.814	1.000	.581	1.000	.897
106	.000	.000	.000	.013	.000	.000	.000	.000	.000	.186	.000	.008	.000	.000
107	.000	.000	.000	.000	.000	.000	.114	.468	.000	.000	.000	.411	.000	.103
OPDH														
105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
106	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
110	.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
111	.000	.000	.000	.000	1.000	1.000	.203	.677	.000	.105	.000	.457	.821	.000
114	.000	.000	.000	.000	.000	.000	.797	.290	.983	.895	.839	.543	.054	.015
118	1.000	.000	.000	.000	.000	.000	.000	.032	.017	.000	.161	.000	.125	.985
IDHF														
97	.000	.000	.000	.000	.000	.000	.000	.032	.000	.000	.177	.000	.000	.000
100	1.000	1.000	.989	1.000	1.000	.800	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.011	.000	.000	.000	1.000	.968	.952	1.000	.823	1.000	1.000	1.000
108	.000	.000	.000	.000	.000	.000	.000	.000	.048	.000	.000	.000	.000	.000
110	.000	.000	.000	.000	.000	.200	.000	.000	.000	.000	.000	.000	.000	.000
PGM														
104	.000	.000	.076	.000	.097	.000	.014	.371	.371	.500	.550	.000	.069	.029
102	.984	.968	.924	.000	.903	1.000	.986	.274	.500	.500	.450	.625	.741	.971
100	.016	.032	.000	.900	.000	.000	.000	.355	.129	.000	.000	.275	.190	.000
98	.000	.000	.000	.100	.000	.000	.000	.000	.000	.000	.000	.100	.000	.000
IDHS														
100	.000	.000	.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
102	1.000	1.000	1.000	.000	.000	.000	.000	.000	.016	.000	.000	.000	.000	.000
104	.000	.000	.000	.000	.000	.000	1.000	1.000	.984	1.000	1.000	1.000	1.000	1.000
OLDH														
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.968	1.000	1.000	.977	1.000	.265
104	.000	.000	.000	.000	.000	.000	.000	.000	.032	.000	.000	.023	.000	.735
SDH														
100	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
104	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
XDH														
97	1.000	1.000	1.000	1.000	.000	.917	.392	.048	.000	.000	.000	.508	.683	.000
98	.000	.000	.000	.000	1.000	.083	.608	.952	1.000	1.000	1.000	.492	.317	1.000
Het	0.050	0.006	0.016	0.069	0.018	0.052	0.102	0.211	0.150	0.099	0.113	0.198	0.146	0.053



Tim	**.755**												
Vind	**.753**	**1.00**											
Prx3	.557	.490	.488										
Prx2	.470	.632	.631	**.917**									
Prx1	.411	.716	.714	**.661**	**.585**								
Oge	.245	.337	.337	.525	.479	.563							
Apa	.233	.319	.317	.446	.452	.490	**.918**						
Mut	.226	.274	.272	.435	.422	.506	**.914**	**.956**					
Boyk	.139	.197	.199	.383	.464	.459	**.873**	**.896**	**.955**				
Cael	.148	.236	.238	.346	.432	.474	**.862**	**.935**	**.879**	**.883**			
Catn	.150	.243	.247	.366	.453	.456	**.859**	**.899**	**.841**	**.856**	**.989**		
Darw	.142	.234	.238	.344	.430	.461	**.846**	**.949**	**.893**	**.910**	**.969**	**.934**	
Vien	.118	.207	.206	.316	.392	.373	**.804**	**.852**	**.804**	**.803**	**.813**	**.776**	**.850**
	Flor	Tim	Vind	Prx3	Prx2	Prx1	Oge	Apa	Mut	Boyk	Cael	Catn	Darw

Figure 3. Nei's (1978) unbiased genetic identities among 14 populations of *Pleurocera* are shown below the diagonal, with conspecific comparisons shaded. Above the diagonal is the neighbor-joining network based on Cavalli-Sforza and Edwards (1967) chord distances. Control populations are designated (c): *P. floridensis* (**Flor**), *P. proxima* (**Prx1**), and *P. catenaria* (**Catn**).

with the Georgia. Then the central tendency of the 21 pairwise comparisons among the seven *Pleurocera catenaria* populations sampled from Georgia (median = 0.125, range 0.032 - 0.219) was slightly lower than the sample of 21 pairwise comparisons among the seven *P. catenaria* sampled from the Carolinas (median = 0.131, range 0.000 - 0.330), and much below the 15 comparisons among populations of *P. proxima* (median = 0.273, range 0.091 - 0.619). Our Mann-Whitney *U*-statistic testing for a difference between the $N - 1 = 6$ segments of the Prim network extracted from the Georgia matrix and the $N - 1 = 6$ segments from the Carolinas was $U = 20$, not significant. Our Mann-Whitney test for a difference in central tendency between the 6 Georgia *P. catenaria* comparisons and the $N - 1 = 5$ comparisons of *P. proxima* populations yielded $U = 3$, a very significant value in the direction opposite of our prediction. Thus there is no evidence that Georgia populations of *P. catenaria* are more genetically variable than pleurocerid populations sampled from similar areas.

DISCUSSION

There is some evidence of a geographic component to the divergence shown among the eight populations identified as *Pleurocera catenaria* in Fig. 3, as has been documented previously for *P. proxima* by Dillon (1984). The population most geographically removed (**Vien**) was also the most genetically divergent of the *catenaria* samples, and the pair of populations collected nearest each other (**Catn** and **Cael**) showed the highest genetic identity (I = 0.99). The group of **Boyk**, **Mut**, and **Apa** was also both geographically close and genetically similar. It is interesting to note that the *catenaria* population designated "Yel" by Dillon and Reed was both the most genetically distinctive and the most geographically removed of the eight populations surveyed in 2002. In the present survey, where that same *catenaria* population was renamed **Mut** and its geographic position rendered internal rather than peripheral, its genetic distinctiveness disappeared.

Our results do not, however, support the hypothesis of endemicity by drainage advanced by previous authors (Mihalcik and Thompson 2002). The divergence among the seven Georgia populations we identify here as *Pleurocera catenaria* is not greater than a sample of seven *P. catenaria* from the Carolinas, despite the fact that both sets of populations were drawn from areas of comparable geographic extent, and significantly less than a sample of six populations of *P. proxima* (Fig. 4).

The first modern review of the Georgia Pleuroceridae was offered by Goodrich (1942), who preferred the generic nomen "*Goniobasis*." He recognized two species in Georgia Atlantic drainages, *Goniobasis catenaria postelli* (Lea, 1858) and *G. mutabilis timidus* (newly described by him),

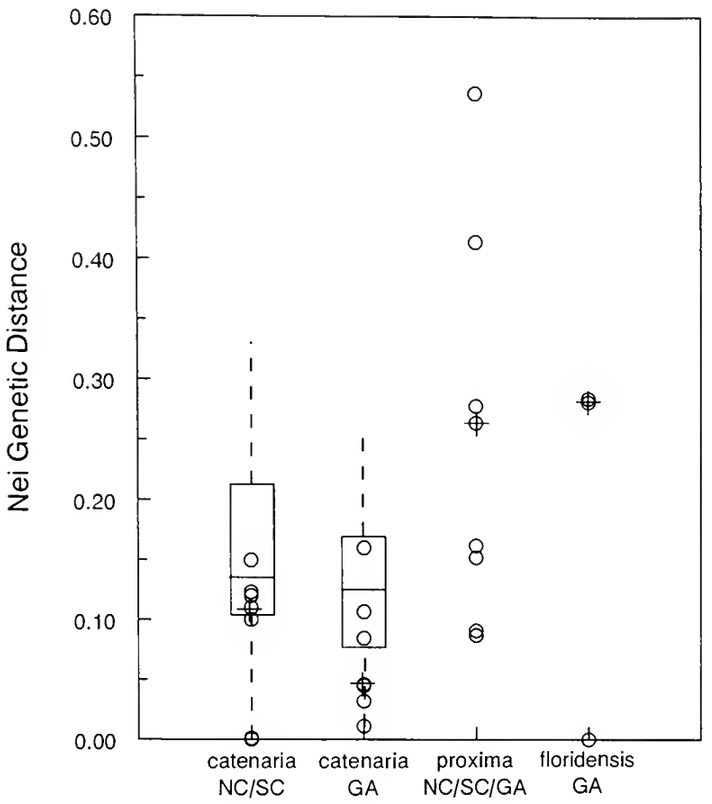


Figure 4. Nei's (1978) genetic distances among all Georgia pairs of *Pleurocera catenaria* from the present study (21 comparisons), *P. catenaria* from the Dillon and Reed (2002) survey of NC/SC (21 comparisons), and *P. proxima* from the 2002 study and the present study combined (15 comparisons). Boxes show medians and quartiles, dashed lines show ranges, and the individual data plotted are the N - 1 segments of the minimum-spanning network.

incorrectly assuming that the range of *G. proxima* extended no further south than the Carolinas. From the Gulf drainages of western Georgia Goodrich recognized seven taxa: *G. catenaria inclinans* (Lea, 1862), *G. catenaria cancellata* (Say, 1829, of which he considered *floridensis* a synonym), *G. boykiniana viennaensis* (Lea, 1862), *G. boykiniana albanyensis* (Lea, 1864), *G. nutabilis* (s.s., Lea, 1862), *G. induta* (Lea, 1862), and *G. curvicostata* (Reeve, 1861). *Goniobasis curvicostata* is a well-characterized species, primarily Floridian in its distribution, not treated in the present work.

Clench and Turner (1956) devoted substantial attention to the Georgia Pleuroceridae in their survey of the freshwater molluscan fauna of American Gulf drainages, recognizing six species: *boykinana* (s.s., Lea, 1840) and *catenoides* (Lea, 1842, extinct), as well as *albanyensis*, *floridensis*, *viennaensis* and *curvicostata*. The dissertation of Krieger (1977) focused on the Atlantic populations of Georgia, nominally *G. postelli*, *G. boykiniana viennaensis*, and *G. suturalis* (Haldeman, 1840). Chambers (1990) simplified the taxonomy of the pleurocerids inhabiting Georgia's Gulf drainages substantially, preferring *Elimia* over *Goniobasis*. He synonymized *inclinans* under *Elimia floridensis*, *albanyensis* under *E. boykiniana*, and *viennaensis* and *induta* under *E. curvicostata*. Chambers also noted the similarity of *E. boykiniana* from Georgia Gulf drainages to *E. catenaria* populations inhabiting Atlantic drainages of the Carolinas, admitting the possibility that the former might ultimately prove a junior synonym of the latter.

Most recently, Mihalcik and Thompson (2002) have recognized 12 nominal pleurocerid species from central Georgia, with no overlap between the Atlantic and Gulf faunas. From Atlantic drainages they listed *Elimia nutabilis* and *E. timida timida*, as well as *E. caelatura* (Conrad, 1849) and three newly-described taxa: *E. timida exul*, *E. timida nymphaea*, and *E. darwini*. From Gulf drainages they recognized *E. boykiniana*, *E. floridana*, *E. induta*, *E. albanyensis*, *E. viennaensis*, and *E. curvicostata*.

No worker since Goodrich (1942) has proposed that any similarity might exist between the virtually indistinguishable pleurocerid populations of the Gulf and Atlantic drainages of central Georgia, or even between Georgia and South Carolina immediately to the east. But against the apparently ancient evolutionary history of the fauna, the presumption of narrow endemism is difficult to rationalize. Approximately 20 km of low hills separate the main Chattahoochee River (draining toward the Gulf) and tributaries of the upper Ocmulgee River (draining toward the Atlantic) in the vicinity of Atlanta (Fig. 1). Further downstream, the main Ocmulgee River and the main Flint River (draining toward the Gulf) are separated by as little as 40 km of flat topography. A more plausible model would invoke bidirectional biotic interchange or suturing of widespread species in Georgia, *P. catenaria* from the north and *P. floridensis* from the south.

Our data suggest that the following nomina variously applied to central Georgia populations of *Pleurocera* should be considered junior synonyms of *P. catenaria* (Say, 1822): *albanyensis* (Lea, 1864), *boykiniana* (Lea, 1840), *caelatura* (Conrad, 1849), *darwini* (Mihalcik and Thompson, 2002), *nutabilis* (Lea, 1862), *postelli* (Lea, 1858), *suturalis* (Hald, 1840), and *viennaensis* (Lea, 1862). In addition, the following should be considered synonyms of *P. floridensis* (Reeve, 1860): *inclinans* (Lea, 1862), *induta* (Lea, 1862), *exul* (Mihalcik and Thompson, 2002) and *nymphaea* (Mihalcik and Thompson, 2002). Much of this synonymy has previously been suggested by Goodrich (1942) or by Chambers (1990). It is interesting that *P. boykiniana* was considered a "probable synonym" of *P. catenaria* by Pilsbry as early as 1891.

Although populations **Flor**, **Vind**, and **Tim** are genetically similar, the shell morphology of the latter two populations does not match that of the former. The shells borne by snails of population **Flor** are marked with strong sculpture, showing both radial costae and spiral cords as is typical for *P. floridensis* (figured by Chambers 1980, 1990). The shells of **Vind** and **Tim** are weakly costate, largely lacking spiral cords.

This is quite reminiscent of the situation in *P. catenaria*, where typical shells sampled from the central part of the range in Georgia and the Carolinas are strongly sculptured with costae and cords (*e.g.*, population **Vien**, Fig. 2), while populations from the eastern edge of the range bear shells that are nearly smooth (figured by Dillon and Reed 2002).

The extent to which such variation in shell morphology may reflect genetic relationships is not clear. Dillon and Reed (2002) reported that the loss of sculpture in two populations of *Pleurocera catenaria* sampled from the eastern edge of the range in South Carolina appeared to be independent, and possibly due to ecophenotypic plasticity (Urabe 2000). In any case, Goodrich (1942) referred such weakly-sculptured populations to the subspecies *Goniobasis catenaria dislocata* (Ravenel, 1834), applying the nomen *Goniobasis catenaria catenaria* to populations bearing the typical shells with strong costae and spiral cords. By analogy, we suggest that weakly-sculptured populations of *P. floridensis* such as **Vind** and **Tim** should be accorded the subspecific designation *Pleurocera floridensis timidus* (new combination), reserving *P. floridensis floridensis* for populations with strong costae and spiral cords as demonstrated in population **Flor**.

The nomen *timidus*, which Goodrich (1942) originally proposed as a subspecies of *Goniobasis mutabilis*, appears to be the earliest name applied by any author to populations we here recognize as a weakly-sculptured form of *Pleurocera floridensis*. Neither Goodrich nor Mihalcik and Thompson (2002) reported *timidus* populations elsewhere beyond tributaries of the Ocmulgee River in the vicinity of Hawkinsville. Thus our inclusion of *Pleurocera* populations from Flint River tributaries (previously referred to the nomen "*induta*") in a broadened concept of *P. floridensis timidus* represents a substantial broadening of the range of this taxon.

Mihalcik and Thompson sequenced a 385 bp fragment of the mitochondrial CO1 gene amplified from 36 individuals representing 17 nominal species and subspecies of pleurocerids, obtaining 26 unique sequences. Although such data must be interpreted with care (Dillon and Frankis 2004, Dillon and Robinson 2009), the general outlines of the maximum-parsimony tree derived by Mihalcik and Thompson agree with the results of the allozyme analysis we report here. Three broad clusters emerged from their analysis, corresponding to our *P. catenaria*, our *P. floridensis*, and *P. curvicostata* (excluded from the present study). The *catenaria* cluster of Mihalcik and Thompson included *darwini* and *viennaensis*, and the *floridensis* cluster included *timida* and *induta*, as might have been predicted from the similarities of these same populations at allozyme-encoding loci.

North of Atlanta, no more than 15 km of low hills separate the upper Chattahoochee River from the Etowah River, which drains west through the Coosa River of Alabama into the Mobile Basin. The Mobile Basin is home to the greatest diversity of Pleuroceridae in North America, approx. 77 nominal species in four genera (Dillon and Lydeard 1998). Although this fauna has conventionally been considered almost entirely endemic (Lydeard and Mayden 1995), the sequence data of Dillon and Robinson (2009) confirmed that the range of *Pleurocera catenaria* extends from the Chattahoochee into the Etowah River, just as it extends from the upper Ocmulgee to the Chattahoochee (Fig. 1). The development of a better model for the origin of the Mobile Basin Pleuroceridae, recognizing its affinities both with the Atlantic fauna here untangled and with the interior fauna of the Tennessee River to its west (Dillon, in press), will be fertile ground for future inquiry.

Coyne and Orr (2004: 123) considered the phenomenon of allopatric speciation "so plausible that it hardly seems worth documenting. Given enough time, and barring extinction, any pair of geographically isolated populations is likely to evolve reproductive barriers." Against this background, the great wonder is not that there are so many species of freshwater molluscs on earth, but that there are so few. We attribute the "opposite of speciation" in the *Pleurocera* populations of central Georgia, despite apparently great antiquity and extreme geographic isolation, to stabilizing selection.

The springs and streams of the Georgia piedmont, as elsewhere throughout the southern Appalachians, are primarily fed by groundwater of relatively constant temperature and chemical composition. The pleurocerids inhabiting these environments are slow-growing, long-lived, perennial generalists, able to graze organic particles over a great range of size and quality, from living single-celled algae to entire leaves dehiscent from vascular plants (Dillon 2000). They are thus insulated to an unusual degree from climatic fluctuations and short-term global catastrophes. And changes in the longer term, such as continental drift, montane erosion, evolution of seed plants, and diversification of benthic insect competitors have been felt uniformly across the pleurocerid populations of the southern Appalachians, imposing stabilizing selection on their morphology, reproductive biology, life history, and other fitness traits, even as molecular clocks have continued to tick.

Throughout most of the history of evolutionary science, researchers have been drawn to centers of great biotic diversity as "laboratories of speciation." We suggest that the future laboratories of speciation should be centers of great evolutionary stasis, such as we describe here.

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Appendix 1. Locality data and taxonomic notes for the 14 populations of *Pleurocera* in this study. Sample sizes given in parentheses. Catalog numbers listed are for dry-lot voucher specimens deposited in the Academy of Natural Sciences of Philadelphia (ANSP).

Apa – (*N* = 37). Apalachee River at Ga183, North High Shoals, Oconee County, Georgia. This was site APAL of Krieger (1977), identified by him as *Goniobasis boykiniana viennaensis*. 33.8176°N; 83.5036°W. ANSP 422170

Boyk – (*N* = 31). Chattahoochee River, about 1 km N of I-285 bridge, Atlanta, Fulton/Cobb County line, Georgia. This site is called "Cochran Shoals," and is presently in the Chattahoochee River National Recreation Area. Chambers (1990) identified a museum lot from this area as *Elimia boykiniana*. Our collections were made at (approximately) the site designated CHAT by Krieger (1977), identified by him as *Goniobasis postelli*. 33.9048°N; 84.4446°W. ANSP 422171

Cael – (*N* = 31). Savannah River at "Savannah Rapids Pavilion" lock and dam, 4 km N of Augusta, Richmond County, Georgia. Populations of *Pleurocera* from this region have been identified as *Elimia caelatura* by Thompson (2000). 33.5500°N; 82.0391°W. ANSP 422172

Catn – *Pleurocera catenaria catenaria* (*N* = 5). Stevens Creek at SC 23 bridge, 24.6 km WSW of Edgefield, Edgefield County, South Carolina. Same population as 4c of Dillon and Keferl (2000) and McC of Dillon and Reed (2002). 33.7292°N; 82.1826°W. ANSP 422173

Darw – (*N* = 31). Rocky Creek at Lord Road, 7 km SW of Dudley, Laurens County, Georgia. This is the type locality of *Elimia darwini* (Mihalcik and Thompson 2002). 32.4889°N; 83.1206°W. ANSP 422174

Flor – *Pleurocera floridensis* (*N* = 31). Blue Spring by the Withlacoochee River at FL6, 15 km E of Madison, Madison County, Florida. Site 8 of Chambers (1980). This population was also sampled by F. G. Thompson for the karyotype studies of Dillon (1989, 1991). 30.4806°N; 83.2448°W. ANSP 422175

Mut – (*N* = 22). Yellow River below dam at Porterdale, Newton County, Georgia. This is population "Yel" of Dillon and Reed (2002), which we identified as *Goniobasis catenaria postelli*. Krieger (1977) designated this population "YELD" and identified it as *Goniobasis boykiniana viennaensis*. Mihalcik and Thompson (2002: 44) identified this population as *Elimia mutabilis*. 33.5683°N; 83.8910°W. ANSP 422176

Oge – (*N* = 30). Ogeechee River at Ga16 bridge, Jewells Mill, Warren County, Georgia. 33.2956°N; 82.7811°W. ANSP 422177

Prx1 – *Pleurocera proxima* (*N* = 5). West Village Creek at SC 196 bridge, 1 km W of Mountain Rest, Oconee County, South Carolina. This was population "West" of Dillon and Reed (2002) and population P1 of Dillon and Robinson (2009). 34.8604°N; 83.1676°W. ANSP 422178

Prx2 – *Pleurocera proxima* (*N* = 31). Small tributary of Nancytown Creek at Forest Service 591 bridge, 2 km SE of Mount Airy, Habersham County, Georgia. 34.5040°N; 83.4809°W. ANSP 422179

Prx3 – *Pleurocera proxima* (*N* = 30). North Oconee River at White Hall Road, 6 km SW of Lula, Hall County, Georgia. 34.3650°N; 83.7317°W. ANSP 422180

Tim – (*N* = 31). Mile Creek at municipal park by US 129, on the south edge of Hawkinsville, Pulaski County, Georgia. This is the type locality of *Elimia timida nymphaea* (Mihalcik and Thompson 2002). 32.2705°N; 83.4658°W. ANSP 422181

Vien – (*N* = 34). Limestone Creek at McCay Rd., 12 km SW of Vienna, Dooly County, Georgia. This site is inhabited by three biological species of *Pleurocera*. Mihalcik and Thompson (2002) identified the population with a broader shell (matching their figures 125–128 and 133–144) as *Elimia viennensis*. 32.0312°N; 83.9076°W. ANSP 422182

Vind – (*N* = 46). Locality data given above. Mihalcik and Thompson (2002) identified the population with a more slender shell and body whorl proximally smoothed (matching their figs. 29–31 and 34–43) as *Elimia induta*. ANSP 422183

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Patterns of speciation in marine gastropods: A review of the phylogenetic evidence for localized radiations in the sea*

Patrick J. Krug

Department of Biological Sciences, California State University, Los Angeles, California 90032-8201, U.S.A.
Correspondence, P. J. Krug: pkrug@calstatela.edu

Abstract: Modern speciation theory is heavily influenced by Mayr's postulate that prolonged geographical isolation is necessary for differentiated populations to evolve reproductive isolation. Present-day distributions of sister species are consistent with allopatric or peripatric speciation in many terrestrial and freshwater animal groups. However, the oceans present few obstacles to dispersal for marine taxa with planktonic larvae, and sister species are not often split at biogeographical breakpoints in the sea. Theory predicts that disruptive selection on habitat choice or resource use can split a population into divergent ecotypes without physical separation, yet sympatric speciation is still often viewed as improbable. Here, I review phylogenetic evidence from diverse marine gastropods to test Mayr's prediction that recently diverged sister species should not be sympatric over most of their ranges. In contrast to expectations, young sister species are often broadly sympatric in many gastropod groups, suggesting that classical models of allopatric divergence are insufficient to explain marine speciation. I discuss four mechanisms that may contribute to this deviation from predicted biogeographical patterns: transient allopatry along continuous coastlines, rapid evolution of gamete recognition proteins, shifts to non-planktonic development, and ecological divergence. The available evidence argues that patterns of marine speciation depend on complex interactions between geography, life history, and ecology, often resulting in local radiations within a basin or endemic to an island group. Whether selection acts on ecotypes in sympatry or on populations during secondary contact, ecological factors may promote speciation in the sea at smaller spatial scales than expected. I highlight areas for future study to improve our understanding of the forces generating marine biodiversity, and why the geography of speciation may be fundamentally different for shallow-water animals.

Key words: allopatry, biogeography, Mayr, ecological, sympatric speciation

> *"The range of the nearest relative of a given species is usually allopatric... This fact suggests that geographic speciation is the principal, if not exclusive, speciation mechanism in most marine animals."* (Mayr 1954: 16)

An outstanding issue in evolutionary biology is whether the speciation process is fundamentally similar among marine, freshwater, and terrestrial animals (Valentine and Jablonksi 1983, Knowlton 1993, May 1994, Palumbi 1994, Briggs 2006). Mayr (1942, 1963) argued influentially that geographic structuring of terrestrial populations promotes divergence in allopatry and the development of intrinsic reproductive isolation over time. Ranges of terrestrial animals may be fractured by barriers to migration such as rivers and mountains, and island populations are inherently isolated from continental ancestors. Similarly, gene flow among lakes and drainage basins can be low for limnetic organisms with little ability to disperse over land. The fragmented nature of terrestrial and freshwater habitats is consistent with Mayr's observations that in groups such as birds, snails, and butterflies, contemporary ranges of sister taxa are non-overlapping, and supported his postulate that speciation usually proceeds in allopatry. This view has become paradigmatic for marine taxa as well, despite the fact that the ocean has few obvious barriers to genetic exchange, and sister species are not always split by the thermal boundaries that demarcate regional faunas (Mayr 1954, Valentine 1966, Briggs 1974, Palumbi and Lessios 2005).

Mayr initially emphasized the primacy of geographic isolation in the early stages of speciation. However, mid-century studies on cichlids and phytophagous insects suggested that sympatric speciation was a viable model for divergence if novel ecotypes could emerge within a population (Thorpe 1945, Trewavas 1947). Mayr countered that ecology contributes to diversification while populations are spatially segregated and experiencing disruptive selection due to environmental differences between their habitats, such that isolation and differential adaptation go hand-in-hand: "all geographical races are also ecological races, and all ecological races are also geographical races" (Mayr 1947: 280). He held that populations overlapping in space would be segregated

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into different niches because ecological divergence was necessary for relatives to co-exist without competing, but argued that divergence precedes secondary contact.

In contrast, modern studies of speciation have focused on mechanism over geographical context, asking whether reproductive isolation arises as a product of natural selection, sexual selection, or the order and rate at which mutations occur and are fixed (by selection or drift) in isolated gene pools (Howard and Berlocher 1998, Turelli *et al.* 2001, Coyne and Orr 2004, Butlin *et al.* 2008, Schluter 2009). Ecologically based selection can promote pre-mating isolation in several ways, such as partitioning individuals by habitat or time of activity (Feder *et al.* 1994), removing maladapted immigrants before they can interbreed (Nosil *et al.* 2005), or reinforcement favoring assortative mating during secondary contact (Coyne and Orr 1989, 2004, Rundle *et al.* 2000, Servedio and Noor 2003, Rundle and Nosil 2005). Ecologically dependent post-mating isolation can arise when hybrids are unfit in parental environments (Egan and Funk 2009). Molecular evidence for positive selection on reproductive proteins, and on "speciation genes" involved in hybrid sterility or inviability, suggests protein evolution may commonly drive speciation (Swanson and Vacquier 2002, Tang and Presgraves 2009).

Reconstructing the dominant mode(s) of speciation in a given clade requires understanding the spatial arrangement of populations, and the timing of genetic changes that result in reproductive isolation. Determining the phylogeny and biogeography of extant taxa is needed to describe the spatial arrangement of related species, while ecological and breeding studies of putative incipient or recently diverged species can identify mechanisms that act during the early stages of speciation. Here, I review evidence from recent ecological and phylogenetic studies of marine gastropods to evaluate whether the data support Mayr's proposal that speciation in the sea requires prolonged allopatry, and results in non-overlapping ranges of sister species. In the past two decades, molecular phylogenetic studies have provided robust hypotheses of evolutionary relationships that permit tests of Mayr's prediction that genetic distance and range overlap should be positively correlated (Knowlton 1993, 2000). I will examine whether in gastropods, sister species or subspecies are usually allopatric, or frequently co-occur over most of their range. I first discuss four general mechanisms, not mutually exclusive, that may allow species to form in proximity and persist with largely overlapping ranges in the sea.

MECHANISMS THAT MAY RESULT IN SISTER SPECIES WITH SYMPATRIC DISTRIBUTIONS

Transient allopatry

Sea level fluctuations driven by Quaternary climate change may have caused transient allopatry for populations of benthic invertebrates along continental margins, especially in intertidal and shallow subtidal habitats (Valentine and Jablonksi 1983, Reid 1990, Briggs 2006). During periods of low sea level, peripheral isolates may have been trapped in refugia where conditions were tolerable, perhaps enhanced by local adaptation to edge conditions or subtidal escape from stress. Long-separated populations may then have come into secondary contact during interglacials. In concert with ecological and reproductive mechanisms that inhibit gene flow, transient allopatry could produce distinctive phylogeographic patterns for coastal taxa in which closely related species have largely overlapping ranges, but discordant patterns of genetic polymorphism across their range (Marko 1998).

Non-planktonic development

For many marine animals, larval development determines the spatial scale at which gene flow occurs, profoundly affecting phylogeography and macroevolution (Strathmann 1978, McMillan *et al.* 1992, Hellberg 1996, Collin 2001, Jeffery and Emlet 2003). Loss of a planktonic larval stage may thus allow local adaptation and lead to speciation in geographical proximity. Planktotrophic larvae are transported by ocean currents for weeks to months while feeding, and are correlated with greater species longevity, larger ranges, genetically panmictic populations, and reduced adaptation to local conditions (Vermeij 1982, Caley *et al.* 1996, Bohonak 1999, Pechenik 1999). Lecithotrophic larvae can develop without feeding, and disperse for days to weeks (broadcast-spawning taxa), or minutes to days if larvae are released from egg masses or adult brood chambers. In non-planktonic development, juveniles emerge into the parental environment, decreasing the range and geological longevity of a species while promoting local adaptation (Hansen 1978, 1980, Jablonski 1986, Palumbi 1994, Todd *et al.* 1998, Sanford *et al.* 2003, Sanford and Worth 2009). Non-planktonic clades may also experience stronger effects of drift due to reduced effective population size, which could intensify divergence during transient allopatry through fixation of alleles that produce intrinsic isolation (Foltz 2003, Foltz *et al.* 2004). Shifts in development may thus have sweeping ecological and evolutionary consequences for marine taxa, a situation without parallel in terrestrial organisms.

Gastropod species with non-planktonic development accumulate over time in the fossil record, suggesting that lecithotrophic lineages may undergo species selection due to larval type, resulting in accelerated cladogenesis (Hansen 1978, 1980, Jablonski 1986). However, if non-planktonic development has frequent independent origins in a given group, non-dispersive lineages may accumulate over time without an increase in speciation rate (Duda and Palumbi 1999, Collin 2001). Phylogenetic studies offer the chance to test whether taxa with non-planktonic development undergo localized radiations resulting in young, sympatric sister taxa,

contrary to prevailing views of allopatric speciation. If so, loss of larvae may be a "speciation factor" (*sensu* Venditti *et al.* 2010) for some clades, but this has yet to be rigorously tested.

Fast-evolving gamete recognition proteins

The rapid divergence of gamete recognition proteins mediating sperm-egg fusion may allow incipient species of broadcast-spawners to co-exist without hybridizing, if mismatches among species produce pre-zygotic isolation (Palumbi 1994, 1999, Swanson and Vacquier 2002). Positive Darwinian selection, a "change is good" scenario at the amino acid level, has favored replacement substitutions in the bindin protein of echinoids and bivalves (Zigler *et al.* 2003, Moy *et al.* 2008). In vetigastropods, signatures of positive selection have been detected for sperm proteins (Swanson and Vacquier 1995, 1998, Hellberg and Vacquier 2000) and egg receptors (Galindo *et al.* 2003, Aagaard *et al.* 2006). Comparable studies have yet to be done on marine animals with internal fertilization, but gamete proteins rapidly diverge in terrestrial insects and mammals (Wyckoff *et al.* 2000, Swanson *et al.* 2001a, 2001b, 2003). Species-specific fertilization may rapidly arise by selection on proteins regulating sperm-egg recognition, permitting close relatives to persist in sympatry even if similar in niche and spawning behavior.

Ecological divergence

Mayr (1947) argued that both reproductive isolation and ecological divergence evolved while populations were geographically separated and were required for subsequent range overlap to develop. However, selection against ecologically intermediate hybrids can result when differentially adapted isolates come into secondary contact, driving the evolution of pre-mating barriers or post-zygotic isolation (Servedio and Noor 2003, McKinnon *et al.* 2004, Rundle and Nosil 2005, Funk *et al.* 2006, Egan and Funk 2009). Further, theory indicates disruptive selection on host, microhabitat or resource use can split one gene pool into two differentially adapted populations of non-competing specialists, even in the face of gene flow (Orr and Smith 1998, Dieckmann and Doebeli 1999, Kondrashov and Kondrashov 1999, Coyne and Orr 2004, Bolnick and Fitzpatrick 2007, Schluter 2009). Geographical isolation is not a requirement for speciation in such models, and sympatric divergence is greatly intensified if sexual selection acts on a condition-sensitive display trait (van Doorn *et al.* 2009).

Ecological speciation may be particularly likely if the trait under disruptive selection drives host or habitat choice and if individuals mate in preferred surroundings. Otherwise, recombination normally separates a trait and mating preference *for* that trait, inhibiting divergence (Rice 1984, 1987, Rice and Hostert 1993, Via 2001). If habitat choice produces assortative mating by pleiotropy, there is no selection-recombination antagonism, and disruptive selection can further diversify incipient specialists through fixation of habitat-specific alleles (Diehl and Bush 1989, Bush 1994, Kawecki 1997, 1998).

Mayr foresaw such arguments nascent in the insect literature, and argued that recurring colonization of a novel host by the parental population, and back-colonization of the ancestral host, would effect gene flow to an incipient host race and thus hamstring divergence: "ecological isolation, in order to be effective, must be able to prevent the mixing of the to-be-separated populations in spite of dispersal" (Mayr 1947: 276). However, repeated invasion of a novel niche is unlikely if selection favors rapid shifts in reproductive and ecological characters following first colonization, as adapted specialists on the novel host would out-compete migrants from the ancestral host. The fly *Rhagoletis pomonella* formed distinct races on introduced apples, blueberries, and snowberries, with host fidelity and host-dependent selection limiting gene flow with populations on the native host; their parasites may also have speciated in sympatry (Feder *et al.* 1988, Filchak *et al.* 2000, Forbes *et al.* 2009). Pea aphids form distinct races on red clover and alfalfa, and gene flow is limited by habitat choice behavior of dispersing adults (Via 1999, Caillaud and Via 2000). Limnetic morphs of stickleback fish likely formed during secondary invasion of glacial lakes, after initial colonizers evolved into a benthic ecotype (Taylor and McPhail 2000). Barriers to gene flow can thus arise rapidly in response to diverse forms of selection and may produce different biogeographical patterns from those predicted from models of allopatric divergence.

Host shifting may be an important driver of speciation in insects, but divergent host use or microhabitat fidelity remains poorly studied for marine taxa (Frey and Bush 1990, Farrell 1998, Sotka 2005, Poore *et al.* 2008). Host shifts could occur among specialized epibionts such as barnacles that settle selectively on sponges, corals, snakes, or whales (Zann 2002). Commensal relationships may also impose selection for host specialization on marine taxa with a vagile adult stage, such as sponge-dwelling shrimp (Duffy 1996a, 1996b) and ophiuroids (Henkel and Pawlik 2005). Speciation by host shifting may be important for small-bodied and specialized consumers such as opisthobranchs (Faucci *et al.* 2007) and amphipods (Stanhope *et al.* 1993, Poore and Steinberg 1999, Sotka *et al.* 1999, Müller *et al.* 2000, Poore *et al.* 2000, 2008, Sotka 2005, 2007). Habitat partitioning along gradients in subtidal depth or intertidal stress represents another mechanism that could allow two populations to diverge within cruising range, if ecotypes are strongly segregated at small spatial scales (Mercurio *et al.* 1985, Davidson and Haygood 1999, Carlon and Budd 2002, Stillman 2002, Muths *et al.* 2006). However, such ecological associations have yet to be studied within an explicitly phylogenetic framework for marine taxa, to uncover potential drivers of speciation without prolonged isolation.

Substrate selection by larvae could be critical to ecological speciation in the sea, allowing genotypes to segregate at small spatial scales in a superficially well-mixed ocean. Due to their limited size and swimming speed, marine larvae have little control over large-scale movements, yet are remarkably adept at colonizing suitable habitat at small scales in response to habitat-specific chemical cues (Pawlik 1992, Krug 2001, Applebaum *et al.* 2002, Bierne *et al.* 2002, Baird *et al.* 2003). If adults maintain host or site fidelity, disruptive selection on larval habitat choice behavior could be a potent factor underlying ecological speciation. Adult microhabitat choice or homing behavior could also keep gene pools separate in the face of potential gene flow—for instance, allowing lineages of the tidepool copepod *Tigriopus californicus* to remain isolated on particular rocky outcrops for decades (Burton 1997).

INFERRING PATTERNS OF SPECIATION FROM RANGES AND PHYLOGENIES OF EXTANT GASTROPODS

Sister taxa that co-occur and occupy different niches did not necessarily speciate in sympatry. Ecological differences between sister species may have evolved (1) during transient allopatry in distinct environments, (2) by character displacement during secondary contact, or (3) from disruptive selection driving ecotype formation in sympatry. Distinguishing among these scenarios is difficult because ranges shift over time, so present-day overlap may not reflect the distribution of populations when speciation occurred (Barraclough and Nee 2001, Losos and Glor 2003). However, simulations of divergence followed by range shifts indicate that contemporary range overlaps for sister species will have different statistical distributions for sympatric, allopatric, or peripatric speciation (Barraclough and Vogler 2000). Phylogenies of birds, freshwater fish, and insects were consistent with expectations for allopatric speciation or peripatric divergence of range-edge populations, with only one case of fully sympatric, recently diverged sister species in *Rhagoletis* flies (Barraclough and Vogler 2000). The available evidence for terrestrial and freshwater taxa is therefore consistent with Mayr's predictions. Below, I review phylogenies of marine gastropods to see if the distributions of recently diverged species deviate from expectations under a strictly allopatric model, a first step in identifying mechanisms that may promote speciation at different spatial or temporal scales in the sea.

Patellogastropoda

Molecular phylogenies of limpets reveal local radiations within the northeastern Atlantic (*Patella* Linnaeus, 1758, 9 spp.), northwestern Pacific (*Nipponacmea* Sasaki and Okutani, 1993, 8 spp.), and along the southeastern Pacific coast of Chile (*Scurria* Gray, 1847, 12 spp.). Two endemic radiations occurred in New Zealand, producing one clade of 8 *Cellana* Adams, 1869 spp. and a second clade of 13 *Notoacmea* Iredale, 1915 spp., a genus restricted to New Zealand except for 3 Australian species (Goldstien *et al.* 2006, Nakano *et al.* 2009). One recently derived pair of sister species was sympatric even at the microhabitat level in New Zealand. Smaller radiations of limpets were geographically restricted to southern Africa (*Scutellastra* Adams and Adams, 1854, 3 spp. and 5 spp.; *Cymbulla* Adams and Adams 1854, 3 spp.; *Helcion* Montfort, 1810, 3 spp.), and the southeastern Pacific (*Nacella* Schumacher, 1817, 3 spp.) (Nakano and Ozawa 2004, 2007). In *Lottia* Gray, 1833 six pairs of sister species are broadly sympatric in the northwestern Pacific, northeastern Pacific, or Western Australia; however, many other sister species remain allopatric (Nakano and Ozawa 2007).

Patterns of speciation may be different for temperate versus tropical lineages (Paulay and Meyer 2002). For instance, among 18 species of *Patelloida* Quoy and Gaimard, 1834 found in the tropical Indo-Pacific, most lineages are allopatric and restricted to distinct archipelagos (Kirkendale and Meyer 2004). Coastal taxa may therefore exhibit distinct biogeographic patterns compared to the fauna of tropical islands. However, phylogenetic evidence indicates at least some limpets speciate in a manner contrary to the expectations of strict allopatry since close relatives co-occur in most genera and whole clades may be limited to the same region or coastline.

Ecological speciation has long been suggested as a mechanism underlying sympatric diversification in limpets, which vary in their degree of substrate specificity (Test 1945, Kimberly *et al.* 1985). Limpets exhibit microhabitat-associated ecotypes or morphological plasticity, such as the barnacle and rock forms of the overlapping sister species *Lottia digitalis* Rathke, 1833 and *L. austrodigitalis* Murphy, 1978 (Crummett and Eernisse 2007). Other species display specificity for a particular substratum or host, including wood (*Pectinodonta* Dall, 1882; Lindberg 1990), shells of bivalves (*L. pelta* Rathke, 1833; Mercurio 1985) or gastropods (*L. asmi* Middendorff, 1847; Lindberg 1990), brown algae (*L. incessa* Hinds, 1842 on *Egregia* Areschoug, 1876; Choat and Black 1979), and seagrasses (*L. alveus* Conrad, 1831 on *Zostera*, *L. paleacea* Gould, 1853 on *Phyllospadix*; Lindberg 1981, Carlton *et al.* 1991). Ecological study of incipient or recently diverged species could shed light on the potential role of selective factors in promoting reproductive isolation in patellogastropods. Alternatively, population genetic studies of temperate limpet species could also test predictions for speciation by transient allopatry, such as reduced genetic diversity in newly colonized overlap regions compared with peripheral refugia.

Vetigastropoda

Tegula Lesson, 1832

Hellberg (1998) analyzed relationships among 23 of 40 *Tegula* spp., a genus about 15 million years old. McLean (2007) recently moved the temperate species *T. brunnea* Philippi, 1848, *T. montereyi* Kiener, 1850, and *T. funebralis* Adams, 1855 to *Chlorostoma* Swainson, 1840 (previously a subgenus) due to differences in columellar morphology between tropical and temperate species. However, according to phylogenetic analyses in Hellberg (1998), this would render *Chlorostoma* either polyphyletic or paraphyletic with respect to *Tegula*. Pending a major revision of this group, I retain the use of *Tegula* for discussion of Hellberg (1998); however, his conclusions about the geography of speciation were based on relationships of temperate species and should be unaffected by the above-mentioned taxonomic changes.

Phylogenetic evidence argued against vicariant severance of connections across the north Pacific, instead revealing separate radiations on each side of the basin. Three clades in the north Pacific were restricted to single coastlines in California, Baja California, and eastern Asia. Five of six sister species pairs were broadly sympatric. Sister taxa were not separated by apparent biogeographical barriers such as the East Pacific deep water, north Atlantic cold water, Isthmus of Panama, or Point Conception, California. The monophyly of sympatric clades was not an artifact of incomplete taxon sampling, as all north Pacific *Tegula* spp. were included. The lone example of allopatric sister species concerned a pair found along the outer coast of Baja and in the upper Sea of Cortez. However, this pair may have had overlapping ranges until closure of the cross-Baja seaway 1 mya; further, they are the most genetically distant pair, which is the opposite of Mayr's prediction that closest relatives should be allopatric.

What fueled diversification along coastlines in *Tegula*? Larval development cannot be invoked, as *Tegula* spp. are broadcast spawners with a moderate larval period of 5-13 days. For taxa along continental margins, sea-level fluctuations may commonly yield overlapping distributions if secondary contact is an inevitable consequence of range expansion after transient allopatry—a "nowhere to hide" scenario (Valentine and Jablonski 1983, Reid 1990, Hellberg 1998). However, Pleistocene range shifts did not sunder one pair of sympatric *Tegula* spp. along the eastern Pacific (*T. brunnea* and *T. montereyi*). The sperm protein lysin undergoes rapid evolution by positive selection in *Tegula*, which may allow young species to coexist if fertilization blocks arise quickly in peripheral or transient isolates (Hellberg and Vacquier 2000). Alternatively, ecological speciation in sympatry may have occurred through microhabitat specialization and differential adaptation to gradients in the physical environment of the rocky intertidal (Riedman *et al.* 1981, Tomanek and Somero 1999, 2000, Tomanek 2002).

Astralium rhodostomum Lamark, 1822 (Turbinidae)

A different pattern of lineage distribution was described for the widely distributed tropical snail *Astralium rhodostomum* (Meyer *et al.* 2005). A phylogeographic study of this nominal species uncovered two clades broadly sympatric across the Indo-Pacific, each containing 30 discrete mitochondrial lineages restricted to a particular island. No appreciable gene flow occurred over as little as 180 km, likely due to a larval period lasting only a few days. The persistence of young lineages with restricted and allopatric distributions is consistent with Mayr's paradigm although the two cryptic species (Clade A and B) described by Meyer *et al.* (2005) were widely sympatric across the tropical Indo-Pacific. Without knowing when reproductive isolation evolves in the lineage sorting process, it remains difficult to assess whether biologically "good" species of turbinids evolve in strict allopatry or parapatry, obscuring the geography of speciation. However, taxa native to tropical archipelagoes exhibit genetic patterns that are different from those living on north-south coastlines (Paulay and Meyer 2002).

Haliotis Linnaeus, 1758

Abalone are broadcast-spawning vetigastropods in which closely related species often co-occur. Seven *Haliotis* spp. are found in California, partly or broadly overlapping in ecological niche, depth zonation, and breeding season. Hybridization can be forced in the lab at high sperm concentration but rarely occurs in the field. Phylogenetic analyses indicate one radiation of 4 species and an independent origin of a further two sister species in California, with additional radiations in Japan (3 species) and Australia (6 species) (Lee and Vacquier 1995, Yang *et al.* 2000). This taxon therefore shows a pattern opposite to that expected under allopatric divergence: close relatives occur in sympatry, and more distant relatives are predominantly allopatric.

Abalone thus represent a conundrum: how do sympatric lineages maintain species integrity when gametes are shed into the ocean at the same time of year? Sperm chemotaxis is species-specific in abalone, but explains only a fraction of the pre-zygotic isolation between two species (Riffell *et al.* 2004). The biochemistry of fertilization has been well studied in abalone, offering a mechanistic understanding of gamete interactions (Swanson and Vacquier 2002). The soluble protein lysin is released from the sperm acrosome and binds to an egg receptor (VERL), causing dissolution of the egg envelope; lysin binding is highly species-specific, and lysin protein sequences diverge rapidly among species (Swanson *et al.* 2001c). The sperm protein sp18 mediates gamete fusion, and its coding sequences are also under strong positive

selection (Swanson and Vacquier 1995). Both proteins are among the fastest evolving genes known, with substitution rates 20 times higher in coding than noncoding sequences (Metz *et al.* 1998). Much of VERL evolves neutrally by concerted evolution, but the amino-terminal end diverged rapidly among species by positive selection (Swanson and Vacquier 1998, Galindo *et al.* 2003). Signatures of adaptive molecular evolution were also detected in 6 of 10 proteins containing zona pellucida domains from the abalone egg jelly coat (Aagaard *et al.* 2006).

Comparison of 25 *Haliotis* spp. revealed that lysin is under strong selection for amino acid substitutions, but replacement rates vary among lineages (Yang *et al.* 2000). Non-synonymous changes accumulated most rapidly among sympatric lineages; rates of molecular evolution were lowest along branches separating distantly related or allopatric lineages. These findings are consistent with the postulate that reproductive isolation can evolve rapidly via selection on gamete recognition proteins among sympatric species. The analysis could not distinguish whether accelerated protein evolution was a consequence of sympatry or a precondition allowing related species to co-occur. However, radiations along a coastline appear to be common in the marine realm, even with little ecological diversification, provided there are other mechanisms by which selection can drive reproductive isolation.

Caenogastropoda

Calyptraeidae

Collin (2003) produced a well-resolved phylogentic hypothesis for 84 ingroup taxa comprising distinct species or divergent populations of calyptraeid slipper shells, a group of sessile, filter-feeding caenogastropods. Of 29 identified sister-species pairs, 11 are sympatric, and all recently diverged sister species (pairwise Bayesian distance of <5%) are either fully sympatric or in close geographic proximity. A further 10 clades (3-9 species each) are found along a single coastline, including species with overlapping ranges. Overall, 72% of sister species are in close proximity to their nearest relative(s) in the absence of any major barrier, with none separated by the Isthmus of Panama, the eastern Pacific deep water, or the Benguela upwelling.

Despite the prevalence of local speciation in the calyptraeids, only one large radiation was restricted to a single region, a clade of eight species with non-planktonic development in the northeastern Pacific (Collin 2004). Non-dispersive development likely fueled speciation in this localized radiation. Outside of this clade, however, only one pair of sister species shared non-planktonic development; thus, reduced dispersal is not generally responsible for co-occurrence of sister taxa in the Calyptraeidae. It is unclear what factors contribute to the high frequency of sympatric sister species throughout the slipper shells. All calyptraeids are sessile filter-feeders, so shifts in trophic ecology are not a viable explanation. Collin proposed transient allopatry or microhabitat partitioning as possible explanations, but further study is needed.

Cypraeidae

From a phylogenetic perspective, cowries are one of the most rigorously studied and well-sampled groups. Meyer (2003) identified evolutionarily significant units (ESUs) as unique mitochondrial lineages that correspond to species, subspecies, or regional populations with a long history of isolation. The majority of cypraeid biodiversity lies in the tropical Indo-West Pacific (IWP), where 12 low-diversity lineages contain 1-3 widely distributed species lacking phylogeographic structure; a few have peripheral endemics. Six lineages contribute 5-26 allopatric ESUs apiece, most showing reciprocal monophyly between basins, concordant with Mayr's proposal that recently evolved sister groups be non-overlapping.

A single lineage, the Erroneinae (130 spp.), represents over half the IWP species diversity in the Cypraeidae. Most species have small ranges and show reciprocal monophyly between basins; only *Talostolida pellucens* Melvill, 1888 has an extensive range with no genetic structure. Based on protochonch morphology, Meyer proposed this group has reduced planktonic dispersal potential but retains a swimming larval stage. Limited potential for dispersal and allopatric diversification among archipelagos likely accounts for the species diversity in this group, as in the turbinid *Astralium rhodostomum*; over half the divergence events in the IWP were allopatric, based on the retained signal of geographical speciation in extant taxa. Peripatric speciation is implicated by the presence of numerous paraphyletic peripheral isolates at the edge of a parent species' range, consistent with theory that this is a common mode of speciation in diverse taxa (Barraclough and Vogler 2000).

Despite the clear evidence for allopatric speciation in the tropics, cowries also illustrate how loss of a dispersive larval stage may spur local cladogenesis. Non-planktonic development evolved at least five times in the Cypraeidae, always on upwelling coastlines such as southern Australia (*Zoila* Jousseaume, 1884, *Umbilia* Jousseaume, 1884, *Austrocypraea* Cossmann, 1903, and *Notocypraea* Schilder, 1927), southern Africa (*Barycypraea* Schilder, 1927, *Cypracovula* Gray, 1824), and the northern coast of Columbia and Venezuela (*Muracypraea* Woodring, 1957) (Meyer 2003). Radiations along a single coast occurred in three Australian genera (*Zoila*, 11 spp.; *Umbilia*, 3 spp.; *Notocypraea*, 5 spp.) and the South African genus *Cypracovula* (14 spp.). Loss of larval dispersal

may simply reduce the spatial scale at which allopatric divergence occurs, or could magnify the effects of local selection and facilitate ecological speciation (see *Littorina*, below). Further study of ecological differences among species in these local endemic flocks is therefore warranted.

Conus Linnaeus, 1758

The hyper-diverse genus *Conus* contains >500 species of predatory cone snails, mostly tropical with 60% of species found in the IWP (Kohn and Perron 1994, Röckel *et al.* 1995). Most species are specialized to feed on either fish, molluscs, worms, or hemichordates, injecting paralytic peptides (conotoxins) through a hollow radula (Kohn and Perron 1994). The degree of specialization on subtypes of the four general prey categories varies among cone snails, but if disruptive selection acted on resource use, cone snails could be candidates for ecological speciation. However, a phylogenetic analysis of 76 *Conus* spp. revealed instead that the same prey type is shared by all unambiguous sister species and often across larger clades (Duda *et al.* 2001). The ancestral condition was likely worm-feeding with 2-4 independent origins of fish predation and one switch to mollusc predation (Duda and Palumbi 2004). Leviten and Kohn (1980) also reported no ecological differences in microhabitat resource use among co-occurring *Conus* spp.

Despite the lack of evidence for speciation by diet shift, a subsequent phylogenetic study of 138 *Conus* spp. revealed that many sister taxa are broadly sympatric in regions such as the Caribbean (*C. lorenzianus* Dillwyn, 1879 + *C. spurius* Gmelin, 1791; *C. mindanus* Hwass in Bruguière, 1792 + *C. jaspideus* Gmelin, 1791; *C. daucus* Hwass in Bruguière, 1792, *C. villepinii* Fischer and Bernardi, 1857 + *Conus* sp. WA-1), the eastern Pacific (*C. lineolatus* Valenciennes, 1832 + *C. princeps* Linnaeus, 1758; *C. bartschii* Hanna and Strong, 1949 + *C. brunneus* Wood, 1828), and eastern Africa (*C. ateralbus* Kiener, 1845 + *C. cuneolus* Reeve, 1843) (Duda and Kohn 2005). The IWP also holds numerous sympatric sister species or radiations, including two sister pairs of worm-feeders (*C. chaldaeus* Röding, 1798 + *C. ebraeus* Linnaeus, 1758; *C. coffeae* Gmelin, 1791 + *C. tennistriatus* Sowerby, 1858), a clade of six worm-feeders, and a clade of seven fish-eaters. Some closely related sister taxa (*C. frigidus* Reeve, 1848 + *C. sanguinolentus* Quoy and Gaimard, 1834 + *C. eburneus* Hwass in Bruguière, 1792 + *C. tessulatus* Born, 1778) specialize on different types of worms, offering the possibility that disruptive selection on resource use fueled divergence although ecological character displacement after secondary contact is also viable.

Perhaps the most dramatic example of a marine "species flock" (a highly localized radiation) is the diversification of *Conus* in the Cape Verde islands off the western coast of Africa. Of 50 *Conus* spp. in the archipelago, 47 are endemic and fall within one or two clades, pending resolution of phylogenetic ambiguity (Duda and Rolan 2005). The radiation in Cape Verde was so recent that some species cannot be distinguished at the fast-evolving mitochondrial cytochrome *c* oxidase I locus, but are ecologically or morphologically distinct. Most species are restricted to one or two islands, and all but *C. damottai galeao* Rolán, 1990 are sympatric with their sister taxon. Rapid cladogenesis on Sal Island resulted in a clade of nine recently diverged species. All 47 Cape Verde endemics have non-planktonic development, further evidence that loss of a planktonic larval stage is correlated with local endemic radiations.

Overall, phylogenetic analyses reject both extreme models of cone snail evolution—that sister species will either be allopatric, or sympatric but differing in resource use. In fact, most cone snails overlap in range but also in prey type with their sister taxon. Future studies of ecology and phylogeography are needed to explore the mechanism(s) behind cone snail diversity. Adaptive evolution of amino acid sequences of the conotoxins offer potential insight into ecological specialization at the molecular level in this group (Remigio and Duda 2008).

Echinolittorina Habe, 1956

The circumtropical genus *Echinolittorina* is restricted to rocky shores, with maximum diversity in the tropical IWP (Williams and Reid 2004). Larvae develop during a four-week planktonic period. Of 59 ESUs identified by genetic analysis, sister species are mainly allopatric, including 7 of 8 pairs in the IWP and 8 of 12 in the eastern Pacific/Atlantic (Williams and Reid 2004). At deeper nodes, about two-thirds of sister clades are geographically partitioned among ocean basins. As predicted by Mayr, the percentage of range overlap is positively correlated with genetic distance in the IWP, where there were no overlapping sister species in archipelagos. Most peripheral IWP species are range-restricted endemics. Most if not all sister taxa are also allopatric in the Caribbean. In contrast, four pairs of recently diverged sister species are broadly sympatric along an eastern Pacific coastline, and one such pair occurs in Asia (Williams and Reid 2004).

The long-term persistence of allopatry in this group may reflect a planktonic larval duration long enough to impede local adaptation, which may otherwise accelerate divergence in transient allopatry or sympatry. Williams and Reid (2004) also proposed that differences between continental shelf versus oceanic island habitats may act as a barrier to dispersal, along with stretches of inhospitable coastline lacking suitable rocky habitat. Different patterns in the IWP and Caribbean versus the coastal Eastern Pacific support the contention that speciation patterns differ between archipelagos and continental margins (Paulay and Meyer 2002).

Nucella Röding, 1798

Dog whelks in the genus *Nucella* comprise six northern Pacific and one northern Atlantic species (Collins *et al.* 1996). All species have non-planktonic development with crawl-away juveniles and are restricted to rocky intertidal habitats. Three members of one clade (*N. lamellosa* Gmelin, 1791, *N. canaliculata* Duclos, 1832, and *N. ostrina* Gould, 1852) are sympatric over most of their ranges from Alaska to northern or central California (Collins *et al.* 1996, Marko and Vermeij 1999, Marko *et al.* 2003). The southern *N. emarginata* Deshayes, 1839, ranging from central California to Baja California, Mexico, shares a recent ancestor with *N. ostrina*, and their mitochondrial haplotypes have not completed lineage sorting. These sister species overlap throughout central California but are habitat partitioned, with *N. ostrina* found in more wave-exposed sites and *N. emarginata* in shallower, less disturbed sites (Marko 1998). Central California populations of *N. emarginata* have reduced genetic diversity at allozyme and mitochondrial loci, suggesting a northward range expansion from a high-diversity Baja population in the late Pleistocene. Similarly, populations of *N. ostrina* from central California have higher diversity and more genetic structure than in the northern half of the range, suggesting a recent expansion of *N. ostrina* along Canadian and Alaskan shores (Marko 2004). In contrast, *N. lamellosa* retains significant genetic structure and diversity across its range, indicating a history of persistence in northern glacial refugia (Marko 2004).

Together, genetic data suggest the deeper-dwelling *Nucella lamellosa* survived in Alaska and Canada during periods of low sea level and cold temperatures, when *N. ostrina* was likely restricted to central California and *N. emarginata* to the Baja Peninsula. Range contractions due to climate change may thus have trapped isolates long enough for reproductive isolation to arise, followed by range expansions that led to secondary contact and present-day sympatry. Population genetic data can therefore provide valuable insight into historical demographics and range dynamics, and can be used to test hypotheses about mechanisms of speciation along north-south coastlines.

Littorina Férussac, 1822

A group of ecologically well-studied gastropods, *Littorina* (19 spp.) are found on rocky shores in the northern Pacific and northern Atlantic (Reid 1996). Phylogenetic hypotheses based on morphology and gene sequences for three loci were largely congruent (Reid *et al.* 1996). In the northern Pacific, 2-3 sister species pairs (*L. brevicula* Philippi, 1844 + *L. mandshurica* Schrenk, 1861; *L. sitkana* Philippi, 1846 + *L. horikawai* Matsubayashi and Habe in Habe, 1979; and likely *L. aleutica* + *L. natica*) have overlapping range edges but are not broadly sympatric. In contrast, sisters *L. squalida* Broderip and Sowerby, 1829 and *L. littorea* Linnaeus, 1758 are fully allopatric (Reid *et al.* 1996). In the eastern Pacific, sister species *L. scutulata* Gould, 1849 and *L. plena* Gould, 1849 are broadly sympatric along California. Phylogenies of Pacific *Littorina* are consistent with vicariance induced by climate change causing peripheral isolation in range-edge refugia, and speciation in transient allopatry (Reid 1990).

In the Atlantic, there are two clades each composed of sympatric species. *Littorina obtusata* Linnaeus, 1758 and *L. fabalis* Turton, 1825 co-occur on both northern Atlantic coasts, while *L. compressa* Jeffreys, 1865, *L. arcana* Ellis, 1978, and *L. saxatilis* Olivi, 1792 are sympatric in the northeastern Atlantic. It is not clear how vicariance could produce the observed species distributions in the northern Atlantic; however, ecological speciation provides an alternative explanation (see below). Further, these five northern Atlantic species all have non-planktonic development, an additional factor that could contribute to the concentration of related species in this region (Reid *et al.* 1996).

Littorina saxatilis has emerged as a model organism for ecological speciation studies (Johannesson 2001). Development is non-planktonic, and adult migratory ability is limited to a few meters per month (Johannesson *et al.* 1993). Within populations, there are steep microclines in morphology and genetics along vertical tidal gradients. Differential selection favors large, banded, and ridged shells for the ecotype living among barnacles in the upper tidal zone; a smaller, smooth morph predominates in the low-water mussel zone (Johannesson *et al.* 1993). In Sweden, hybrid snails with intermediate characters occur in barnacle-to-mussel transition zones, where they may have a fitness advantage over pure parental types (Janson 1983). In Spain, hybrids are rare and selection favors different alleles of the aspartase amino-transferase enzyme across a three-meter transition zone (Johannesson *et al.* 1995a).

In Spain, three factors underlie reproductive isolation between the morphs (Johannesson *et al.* 1995b). First, pure ecotypes aggregate through microhabitat choice, clustering together even in the mid-shore transition zone where available habitat is a mosaic of mussel and barnacle patches. Thus, the two morphs do not encounter each other frequently, even when intermingled mid-shore. Snails also aggregate by size within morphs at all shore levels. Second, pairs mate assortatively in the field, even after controlling for micro-distributional bias, indicating a behavioral component of mate choice. Pure-by-hybrid pairs conform to a random distribution in the transition zone, however, and mating between pure morphs is also random in the lower zone due to the rarity of the upper-shore ecotype; such cross-morph matings cause limited gene flow between the pure morphs. Third, hybrid females have lower fitness than the upper-shore morph.

It was initially unclear if size-assortative mating was a pleiotropic effect of selection on size due to microhabitat use, or a behavioral response to selection against hybrid offspring. Pure ecotypes survival is highest in their home zone, lowest in the opposite zone, and intermediate in the transition zone (Rolán-Alvarez *et al.* 1997). However, hybrid survival is equivalent at all tidal heights and equal to that of pure morphs in the transition zone. Since hybrids are as fit as pure morphs, assortative mating likely evolved as a pleiotropic effect of selection on other characters and not to selection against hybridization. Genetic studies further suggest that ecotypes evolved in parallel at multiple sites under the same selective pressure (Rolán-Alvarez *et al.* 2004).

Different conclusions were reached for British *Littorina saxatilis* present as distinct shell morphs on a cliff side versus rock cobble (Grahame *et al.* 2006). Where the two habitats abutted, a cline in shell morphology was congruent with a genetic discontinuity. A sharp break in allele frequencies was detected at 15 loci thought to be under strong selection, and analysis of 275 undifferentiated loci supported a general barrier to gene flow across the habitat cline. Grahame *et al.* (2006) argued this pattern reflects allopatric divergence, secondary contact, and introgression. They disputed parallel divergence of Spanish *L. saxatillis* on the grounds that rare long-distance dispersal and colonization events were alternative explanations for the co-existence of ecotypes (see also Butlin *et al.* 2008).

Spanish hybrid zones in *Littorina saxatilis* are of "primary origin" if they arose in parallel via sympatric divergence of ecotypes; alternatively, the two ecotypes may have evolved one or more times in allopatry, with present-day overlap zones reflecting secondary contact. Quesada *et al.* (2007) sequenced 1.83 kb of mitochondrial DNA to distinguish between these competing hypotheses. Genetic data revealed strong differentiation among four sites with locally restricted haplotype clades, yet many polymorphisms and even haplotypes were shared between ecotypes within a site. Coalescent estimates suggested ecotypes diverged in parallel at each site within the last 40,000 years, as *L. saxatilis* spread south along the Galician coast (Quesada *et al.* 2007). Genetic and experimental evidence in Spain thus support repeated, sympatric divergence of ecotypes due to strong selection across an environmental cline, with assortative mating evolving as a byproduct of selection on traits associated with habitat use.

Sadedin *et al.* (2009) modeled the evolution of ecotypes and assortative mating to explore the range of parameters that favored speciation in the *Littorina* system. In simulations, ecotypes often evolved under ecologically relevant values for the strength of selection, number of loci influencing ecologically key traits, mate choice system, size of the hybrid zone, and source of asymmetry in population density. Ecotype formation was limited not by gene flow, but by selection impeding colonization of alternative tidal zones by one morph, genetic systems where traits were controlled by many loci of small effect, or frequent long-distance dispersal that reduced genetic structure (Sadedin *et al.* 2009). After ecotype formation, symmetric assortative mating evolved often but did not always increase in strength; assortative mating could evolve transiently or persist long term without leading to speciation, especially if selection favored a zone of hybrid superiority like in Swedish *L. saxatilis* (Sadedin *et al.* 2009). However, strength of assortative mating increased over time in some simulations. Thus, depending on the interplay of selection, quantitative genetics, dispersal and mate choice, ecotypes can be an evolutionarily stable state or an intermediate in the process of sympatric speciation via disruptive selection.

Heterobranchia

Chromodorididae

Until recently, the colorful chromodorid nudibranchs and their relatives were thought to comprise a few speciose genera, each with representatives in several ocean basins, and several less diverse genera with restricted distributions. Molecular phylogenic analysis revealed instead that traditional genera are not monophyletic; eastern Atlantic species of *Hypselodoris* Stimpson, 1855 are not reciprocally monophyletic with respect to Indo-Pacific congeners, and species of *Mexichromis* Bertsch, 1977 in the western Atlantic and eastern Pacific are not sister groups (Wilson and Lee 2005, Turner and Wilson 2008). Despite long-standing assumptions about the influence of vicariance on biogeography, sister species of chromodorids are sympatric in many clades, and local radiations have occurred along single coastlines. For instance, *Chromodoris purpurea* Risso in Guérin, 1831 and *C. krohni* Verany, 1846 are sympatric in the eastern Atlantic and Mediterranean, as are four *Hypselodoris* species (*H. bilineata* Pruvot-Fol, 1953, *H. orsinii* Verany, 1846, *H. picta* Schultz, 1836, and *H. villafranca* Risso, 1818). All three known species of *Digidentis* co-occur in southeastern Australia (Turner and Wilson 2008). Numerous radiations also occurred within the tropical IWP.

Because chromodorid nudibranchs can be highly stenophagous, specializing on particular species of chemically defended prey sponges, ecological speciation by resource partitioning is one possible explanation for speciation in sympatry. Further ecological study of nudibranch resource utilization, depth zonation, and larval development may reveal whether sympatric ecological specialization or transient allopatry can explain the co-occurrence of sister taxa. For example, four species of *Chromodoris* (*C. tasmaniensis* Bergh, 1905, *C. epicurea* Basedow and Hedley, 1905, *C. daphne*

Angas, 1864, *C. splendida* Angas, 1864) are sympatric in eastern Australia, but as at least three feed on the same sponge species, ecological divergence is not a viable explanation. Questions worthy of investigation include how close relatives can (1) co-exist without hybridizing, and (2) occupy the same specialized niche without one out-competing the others.

Morphological study suggested that the direct-developing nudibranch *Doris "kerguelenensis"* Bergh, 1884 was one widespread species with a circumpolar distribution around Antarctica (Wägele 1990). However, molecular data revealed an extraordinary radiation of 29 putative cryptic species (17 occurring in Bransfield Strait alone), with rarefaction analysis suggesting many more yet to be found (Wilson *et al.* 2009). Transient isolation of lineages in multiple refugia during Pleistocene glaciation cycles may have fueled this radiation in the Southern Ocean. Non-planktonic development is also implicated as a factor underlying rapid, local cladogenesis in this putative species flock.

Phestilla Bergh, 1874

Nudibranchs in group Aeolidina are specialized predators of cnidarians and show a range of coevolved adaptations to their prey, including sequestration of cnidae (Greenwood and Mariscal 1984, Martin 2003), retention of diet-derived zooxanthellae (Rudman 1981, Burghardt *et al.* 2005, 2008), and crypsis (www.seaslugforum.net/message/13424). Despite the potential for ecological speciation among aeolids, little attention has gone to the role of host shifting in their lineage diversification. Faucci *et al.* (2007) presented a phylogeny of the coral-eating nudibranch genus *Phestilla*. Although no ancestral character state reconstructions were performed, their data suggest that the ancestral *Phestilla* fed on a species of the hard coral *Porites*, as do all extant species except a derived pair of sister species that feed respectively on *Tubastrea* Lesson, 1829 (*P. melanobranchia* Bergh, 1874) and *Goniopora* de Blainville, 1830 (undescribed *Phestilla* sp. 2; Faucci *et al.* 2007). Additional studies are needed to test whether host shifting is a viable mechanism for speciation among aeolid nudibranchs.

Sacoglossa

The Sacoglossa are a clade of small-bodied, mainly herbivorous sea slugs that feed suctorially on large-celled, typically siphonaceous host algae; two genera feed on eggs of other opisthobranchs, and a few species radiated onto marine angiosperms or diatoms (Jensen 1983, 1996, Trowbridge 2002). Most sacoglossans are host restricted, feeding on one algal genus or a preferred species, making the Sacoglossa one of the only groups of specialized marine herbivores (Jensen 1989, Trowbridge 1991, Trowbridge and Todd 2001, Poore *et al.* 2008, Trowbridge *et al.* 2008, Baumgartner *et al.* 2009, 2009, 2010). The radula of a given sacoglossan species may be adapted to the host alga's cell wall, and slugs typically sequester defensive toxins from their host (Cimino and Ghiselin 2009). Members of the speciose clade Plakobranchoidea retain functional chloroplasts from their diet, and a few acquired functional algal genes through lateral gene transfer (Pierce *et al.* 2003, Händeler *et al.* 2009, Schwartz *et al.* 2010). Larvae of some sacoglossans metamorphose in response to host-specific chemical cues, further evidence of coevolution between slug and host (West *et al.* 1984, Krug and Manzi 1999, Krug 2001; but see Trowbridge and Todd 2001).

Sacoglossans thus show specialized associations analogous to those of phytophagous insects and angiosperms. Further, sympatric congeners are often partitioned onto different host algae, making sacoglossans a model system for investigating whether host shifts facilitate speciation. Phylogenetic and ecological studies currently underway suggest that host shifting has occurred pervasively over the evolutionary history of the shell-less sacoglossans, and has been a key driver of diversification in the speciose genus *Elysia* Risso, 1818 (Krug, Rodriguez, Trathen, Ellingson, and Trowbridge, unpubl. data). For example, out of 54 *Elysia* spp. studied to date, the most recently diverged sister species (*E. pratensis* Ortea and Espinosa, 1996 and *E. subornata* Verrill, 1901) co-occur throughout the Caribbean and are sympatric in the same habitat, but are partitioned onto different host algae. Despite a history of hybridization and mitochondrial introgression, they remain distinct in morphology and in their nuclear genome (Rodriguez 2009). Ecological speciation in sympatry is a viable explanation when recently divergent taxa have entirely overlapping ranges yet maintain species integrity despite hybridization due to differential resource use and habitat association. Cycles of niche expansion and contraction, potentially driven by changing tolerance for chemical defenses, may underlie host shifts in sacoglossans as has been proposed for terrestrial insects (Camara 1997, Janz *et al.* 2006).

In some cases, however, sympatric and congeneric sacoglossans may share the same host (Trowbridge *et al.* 2008, 2009, 2010). A robust phylogeny is needed to resolve whether co-occurring species are generally (a) host-partitioned sister taxa, as predicted by ecological speciation; (b) host-sharing but not closely related, as predicted by Mayr; or (c) host-sharing sister taxa, which are not predicted by either model. The last prediction, if borne out, would suggest that contrary to classical ecological theory, there is little competitive exclusion among these small-bodied herbivores. Ongoing studies will test whether host shifting and host diversity are drivers of speciation in sacoglossans, and will facilitate comparisons between specialized herbivores in marine and terrestrial systems (*e.g.*, Becerra 1997, Cook *et al.* 2002, Janz *et al.* 2006, Leschen and Buckley 2007).

CONCLUSIONS

Phylogenies of diverse marine gastropods show that recently diverged sister species are often sympatric, especially along temperate coastlines, in the Caribbean, and on remote island chains (Table 1). This contrasts sharply with the distribution of sister species among terrestrial faunas, and with predictions under models of allopatry with subsequent range shifts. Taxa with centers of diversity in the Indo-West Pacific, such as cone snails and cowries, exhibit species distributions that are consistent with models of allopatric divergence, but even in these groups endemic radiations occur in peripheral regions. Future tests comparing distributional patterns against statistical null models are therefore warranted for marine animals, especially where plausible alternative models can be invoked, such as ecological speciation in sympatry.

In groups like *Nucella* and *Echinolittorina*, transient allopatry may explain the overlapping ranges of related species along north-south coastlines. Range shifts following peripheral isolation during Pleistocene glacial-interglacial cycles should leave genetic signatures that may allow tests of this model. In broadcast-spawners such as *Haliotis* and *Tegula*, the rapid divergence of gamete recognition proteins among transiently allopatric populations may allow recently diverged or incipient species to co-occur without fusing back into one gene pool. Selection favoring amino acid changes may thus act during secondary contact to confer reproductive isolation, and produce the observed patterns of sympatric overlap. Whether positive selection also drives functional diversification of sperm

Table 1. Summary of distributional patterns of sister species in marine gastropods for which detailed molecular phylogenies exist. Checkmarks indicate whether the majority of studied species broadly overlap with their closest relative or remain allopatric. An "X" denotes evidence for a mechanism that could promote divergence or co-existence of young sister species in sympatry, while a "?" denotes a suggested mechanism for which evidence is lacking. Parenthetical notes refer to specific cases discussed in the text for that group.

Taxon	Sister taxa primarily allopatric	Sister taxa frequently sympatric	Potential mechanism behind sympatric distribution of sister taxa: Transient allopatry	Benthic development	Gamete recognition	Ecological divergence
Patellogastropoda	✓ (tropical)	✓ (temperate)	?			?
Vetigastropoda						
Tegula		✓	X		X	
Turbinidae	✓					
Haliotis		✓			X	
Caenogastropoda						
Calyptraeidae		✓	?	X (one radiation)		? (microhabitat)
Cypraeidae	✓	✓ (peripheral isolates)		X (upwelling coasts)		
Conus	✓	✓ (Cape Verde Is.)		x		
Echinolittorina	✓	4 pairs in E. Pacific				
Nucella		✓	X			
Littorina		✓	X (Pacific)	X		X (Atlantic)
Heterobranchia						
Nudibranchia		✓	X	X (Antarctic *Doris*)		X (*Phestilla*)
Sacoglossa		✓				X (host shifts)

proteins and egg receptors in marine animals with internal fertilization remains an open question for future research.

In most of the groups reviewed here, there are many examples of almost entirely sympatric sister taxa or local radiations that are harder to explain by transient allopatry, including the Patellogastropoda, *Littorina*, *Calyptraea*, nudibranchs, and sacoglossans. The frequent occurrence of sympatric sister species in these groups argues that an ecological basis for speciation needs to be carefully considered for marine animals, before assuming that allopatric divergence explains the bulk of speciation in the sea. In several of these taxa, host or microhabitat associations suggest ecological mechanisms by which disruptive selection may act to sunder a population into specialized ecotypes (Table 1). Reproductive isolation can arise through the diverse mechanisms discussed earlier, allowing close relatives to overlap in range.

Life-history transitions to non-planktonic development may also promote speciation "in proximity," meaning divergence that allows species to form near each other and fast enough to achieve present-day sympatry. Non-planktonic development is associated with local radiations in temperate zones for littorinid and calyptraeaid lineages. Loss of a dispersing planktonic phase is also linked to bursts of diversification in tropical groups, including Cape Verde *Conus* and cypraeids that are peripheral to centers of Indo-Pacific diversity; these groups otherwise show patterns consistent with allopatric speciation across archipelagos. Direct development reduces the spatial scale at which selection acts, increasing local adaptation and likely promoting divergence among ecotypes or populations. The synergistic effects of niche diversification and non-planktonic development should receive attention through comparative studies that control for phylogenetic effects, to test whether this one-two punch indeed promotes speciation.

Although inferring the geography of speciation will remain difficult, phylogenies of marine gastropods illustrate that sister species are broadly sympatric in many groups. This pattern contrasts strongly with theoretical simulations of allopatric divergence and range shifts, and with empirical results for terrestrial and freshwater taxa. Ecological speciation by disruptive selection, potentially facilitated by loss of a planktonic stage, deserves careful attention in efforts to understand the genesis of marine biodiversity. Whether acting during secondary contact or in sympatry, the role of selection must be better studied to explain why close relatives so often co-occur in the shallow waters of our oceans.

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Towards solving Darwin's "mystery": Speciation and radiation in lacustrine and riverine freshwater gastropods*

Matthias Glaubrecht

Museum für Naturkunde, Leibniz Institute for Research in Evolution and Biodiversity at the Humboldt University Berlin, Invalidenstrasse 43, D-10115 Berlin, Germany

Correspondence, M. Glaubrecht: matthias.glaubrecht@mfn-berlin.de

Abstract: As much as the century-long debate on what species are has created confusion and controversy, not less has speciation been discussed, *i.e.*, the process of how new forms and taxa evolve, ultimately resulting in the multiplication of species. Darwin's often cited "*mystery of the mysteries*", *i.e.*, the origin of species and, as a consequence, of biological diversity in general, remains at the forefront of current evolutionary biology. While allopatric speciation by geographical separation is still considered an important mechanism in most cases, recently it became evident (as a slow and quiet revolution) that alternative explanations may account for how new species come into being. Among the most prominent factors, particularly in the context of adaptive radiation, is ecology in concert with specialization, albeit not necessarily in a sympatric setting. Unfortunately, for freshwater gastropods we are far away from really understanding the details of the various speciation processes (allopatric versus sympatric) and the role of natural selection, adaptation and ecology that have lead to the array of radiations described recently for several taxa and cases. Contrasting lacustrine and riverine settings, I will discuss these issues for limnic Cerithioidean gastropods, in particular for (1) paludomids from East Africa (*i.e.*, the thalassoid species from Lake Tanganyika and *Potadomoides* Leloup, 1953 in the Congo River system), (2) pachychilids *Tylomelania* Sarasin and Sarasin, 1897 endemic to lakes on Sulawesi in Indonesia, (3) *Brotia* H. Adams, 1866 in the Kaek River drainage in Thailand, and (4) *Madagasikara* Köhler and Glaubrecht, 2010 on Madagascar. With an increasing armamentarium of molecular genetic techniques for exploring the genetic structure of populations and species, the one mystery has become many, and possible solutions multiplied, as we uncover further complexities in what species really are and how they multiply.

Key words: phylogenetic pattern, fossils, microevolutionary studies, Lake Tanganyika, Congo River, Lake Turkana

"These facts seemed to me to throw some light on the origin of species – that mystery of mysteries, as it has been called by one of our greatest philosophers." Darwin (1859: 1)

Albeit often ignored, Jean Baptiste de Lamarck's (1809) proposal that species change through time and that fossils represent ancestors of living species can be seen as the first consistent theory of genuine evolutionary change (Mayr 1982, Corsi 1988). Although Lamarck's ideas on the origin of species were widely known and discussed, they were largely dismissed. As many biographers have pointed out (*e.g.*, Desmond and Moore 1991, Browne 2002), even Charles Darwin later in his life denied any influence of Lamarck on his own thinking about variational evolution and the very process that brings species about. In his epochal "*Origin of Species*", Darwin (1859) depicted one diagramatic sketch of a hypothetical phylogenetic tree to illustrate and explain, as we understand today, biological diversity as the result of descent with modification and a continuous process leading to a branching pattern, namely speciation as we call it today.

Since then evolutionary trees have become the lingua franca of biology. Throughout the history of systematics and evolutionary biology there has been no shortage of attempts to find a classification system that captures the diversity of living forms in nature (see, *e.g.*, historical review and references in Endersby 2009, Glaubrecht and Haffer 2010). What Charles Darwin (1859) once called the "*mystery of the mysteries*", *i.e.*, the origin of species and, as a consequence, of biological diversity in general, remains at the forefront of current evolutionary biology. Therefore, the fundamental question as to how new species evolve is one of the six "*Darwinian mysteries*", or big questions in evolutionary biology and biodiversity, all still in need of answering (Glaubrecht 2009).

In this context, it is important to realize the two focal points of evolutionary biology, anagenesis, viz. natural selection acting to transform existing taxa, versus cladogenesis, or the actual multiplication of species. Introducing the concept of natural selection, Darwin's work is justifiable marvelled as brilliant induction in presenting insightful

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examples of adaptation and anagenesis. "*I am convinced that Natural Selection has been the main but not exclusive means of modification*", he wrote in the introduction to his book on the "*Origin of Species*" (Darwin 1859: 6). However, Darwin struggled in vain with the question of the multiplication of species or cladogenesis. It was Mayr's (1942, 1963) syntheses, including the formulation of the concepts both of biological species and allopatric speciation, that finally and for long provided an explanation for cladogenesis, grounded chiefly in geography, as a factor largely ignored before.

ON SPECIES AND SPECIATION

Speciation, arguably the fundamental step in cladogenesis, is a key process in evolutionary biology with paramount consequences for systematics, genetics and ecology. Its study, including historical and ecological aspects, is one of the most active research field in evolutionary biology, with the development of testable criteria for distinguishing alternative hypotheses (for the most recent reviews see, *e.g.*, Coyne and Orr 2004, Gavrilets 2004, Mallet 2008a, Nosil 2008, Butlin *et al.* 2009, Schluter 2009).

Most essential in this context is, of course, our perception of species as being of major importance for biology in general and evolutionary studies in particular. The debate on speciation is firmly entangled with the so-called "species-problem". As, for example, the biological species concept is explicitly related to the process of speciation, this and other concepts determine how speciation is studied. Coyne and Orr (2004) have shown how to distinuish alternative hypotheses about this process. The question of delimiting vs. defining species is currently seeing a remarkable renaissance; yet, no consensus on the nature of species has been reached, and intensive debates about species concepts continue. For a discussion of the frequent confusion of species concepts, species categories and the species taxon see, *e.g.*, Bock (2004). For the most recent reviews on the species problem, see Hey *et al.* (2003), Sites and Marshall (2003, 2004), Coyne and Orr (2004), Glaubrecht (2004, 2009), Heuer (2008), Endersby (2009), Wilkins (2009), and Richards (2010). As Noor (2002) pointed out correctly, none of the recently suggested, lineage-based species concepts have facilitated research on the process of speciation like the biological species concept before. Only when we regard species as natural entities that have a real existence in nature, and are defined using the reproductive criterion, is the study of mechanisms of speciation a useful and potentially informative endeavour.

As much as the century-long debate on what species are has created confusion and controversy, not less has speciation been discussed as ultimately resulting in the multiplication of species. Consequently, speciation remains at the forefront of evolutionary biology. Most generally, speciation is understood here as being caused by a change in environment, be it the establishment of geographical isolation (*i.e.*, allo- / para- and peripatry) or the onset of divergent selection in an ancestral population by sympatric speciation through ecological specialization. Barraclough (2004) has pointed out, in criticising some theoretical speciation models, that to date the role of environment has been merely considered in fixed or simple terms, thus underestimating its importance in speciation. In order to provide biologically relevant models, we need to study realistic model organisms, looking in detail into the dynamics of the underlying mechanisms of speciation and radiation. These studies involve possible links between geographical patterns and ecological processes of speciation, as well as the potential role of introgression and hybridization, and use evolutionary branching and populations genetics in a variety of spatially structured and specialized populations, combined with correlated morphological data and ecological observation, to estimate the environmental influence on speciation.

I propose that in particular those freshwater molluscs and their specific insular environments discussed in the second part of the present paper provide suitable models for this kind of study. Prior to that I will briefly review the different modes and models of speciation and hint at the relevant literature on this subject, providing an overview of our knowledge for limnic gastropods.

MODELS OF SPECIATION

"*One could not fully understand new theories unless one understood the history of the subject and the nature of the displaced theories.*" Mayr (1999: 402)

It has been proposed that in biology concepts play an equally important role as do natural laws in physics and chemistry (Mayr 1997). Given their heuristic significance, biological concepts, such as allopatric, intra-lacustrine or ecological speciation, as well as adaptive radiation or coevolution and competitive displacement, need case studies for evaluation and illustration of their general validity. Our current understanding of how species multiply was influenced by Mayr's (1942, 1963, 1982, 2001) credo that geographic distribution and spatial isolation play a key role in allopatric speciation, and in the modern synthesis of evolution it became firmly established that geographical separation actually is a major factor in speciation (*e.g.*, Coyne and Orr 2004, Mallet 2008b, Cain and Ruse 2009).

Glaubrecht (2002, 2007a, 2007b) has outlined the historical avenues and intellectual roots of particularly the contributions of German systematists of the "Berlin School", built by Erwin Stresemann, Bernhard Rensch and Ernst Mayr and their "new systematics", regarded as crucial for having provided in the 1920s and 1930s the foundation for the

synthetic evolutionary theory. Summaries of knowledge on speciation were given first by Rensch (1939) and Mayr (1940). This perception of the role of the "Berlin School", though, is largely contrary to that of, for example, Junker and Hoßfeld (2002) and Mallet (2004), who largely ignored Stresemann's strong influence on the roots of Mayr's thinking. However, as Cain (2009) has pointed out, it is necessary to rethink our understanding of the evolutionary synthesis. In case of Mayr at least, this would bring species again back as focal point of the evolutionary process and help to better understand the history of speciation theory, or what Mayr (1947: 263) termed "the gradual formulation of the theory of geographical speciation".

Without doubt, modern molecular data from phylogenetic, phylogeographic and genomic studies have revealed a greater complexity of pattern and process than Mayr (1942) and subsequent researchers could ever have expected (*e.g.*, Emerson 2008, Venditti and Pagel 2009, Venditti *et al.* 2010, Turner and Hahn 2010, for a recent exemple of our own species, see Presgrave and Yi 2009). In understanding evolution it has also helped to view speciation not as an endpoint but as a process. However, with the focus on factors involved in or engines powering this process, speciation is still a highly contentious issue (Hendry 2009), with allopatric versus sympatric speciation remaining one of the great controversies in evolutionary biology.

Allopatric speciation

Speciation in bisexual organisms depends on the disruption or strong limitation of gene flow between the populations involved. The simplest way to achieve this is indeed by geographic separation, with allopatric divergence being produced either by genetic drift or selection in different environments. Many observations lead to the model of allopatric speciation by Mayr (1942, 1963, 2001). For recent reviews of the various stages, see *e.g.*, Allmon (1992) and Grant and Grant (2008).

For six decades, allopatric speciation has been the dominant concept, as the theoretical possibility of reproductive isolation in allopatry is undebated, and examples are plentiful (reviews, *e.g.*, Barraclough and Vogler 2000, Grant 2001, Salomon 2001, Via 2001, Losos and Glor 2003, Coyne and Orr 2004, Nosil 2008, Butlin *et al.* 2009). Under the allopatric speciation paradigm, geography and its consequence, viz. blocking gene flow between populations, is regarded as the sole causation for population divergence and ultimately reproductive isolation, with spatial separation being the key. It allowed Coyne and Orr (2004) to conclude that studying speciation is largely synonymous with studying reproductive isolation. Allopatric speciation was long widely regarded as the parsimonious default or null hypothesis, and geographic mechanisms preventing interbreeding generally thought to be the most common way for new species to arise. For a contrary view, see Bush (1975, 1994, 1998), Wu (2001), Mallet (2001, 2008a, 2008b) and Johannesson (2009). However, the perception promoted *e.g.*, by Schuilthuizen (2001) that the impact of geographical barriers has been trivial rather than paramount is misleading.

Recent advances in phylogeography provide strong support for the importance of geographical isolation in diversification and genetic differentiation. Several well-researched case-studies, *e.g.*, so-called ring species and pairs of closely related species, indicate that incipient species formation is more complex than generally assumed, involving a number of genetically distinct components and various instances such as range contraction, isolation, differentiation, and secondary contact, even on small geographic scales. For examples, see Wake (1997), Peterson *et al.* (1999), Irwin *et al.* (2001, 2005), Grant and Grant (2008, 2009) as well as case studies compiled in Glaubrecht (2010a).

Theoretical aspects of the role of geography in speciation and its detection were explicitly discussed, for example, in Barraclough and Vogler (2000) and Losos and Glor (2003). The unsuspected complexity of allopatric speciation is particularly apparent in a special case, parapatric speciation. Usually involving an ecotone, a sharp ecological (as opposed to geographical) boundary in the distribution range of a species, a number of examples have recently emerged (Johannesson *et al.* 1995, Smith *et al.* 1997, Tatarenkov and Johannesson 1998, Schneider *et al.* 1999). Assortative mating, *i.e.*, non-random mating due to preference for similar partners resulting in increased reproductive isolation, plays an important role, as it also does in sympatric speciation (Malausa *et al.* 2005), and its theoretical aspects have been tested in Gavrilets *et al.* (2000).

Sympatric speciation

While allopatric speciation *sensu* Ernst Mayr is still considered an important mechanism in many cases, over the last two decades alternative explanations as to how new species come into being gained more attention. As a slow and quite revolution, it became evident that an ecological rather than geographical explanation may account for speciation. In fact, as much as allopatric speciation is important in many cases, it certainly is not the only mechanism and might not even be of the eminent importance of which Mayr (2001) was convinced throughout most of his long life.

In this context the model of sympatric speciation, the evolution of reproductive incompatibility in a population with no extrinsic geographic barriers to gene flow, is contentiously discussed. As speciation models largely focus on the spatial component of speciation, the difference to which extent gene exchange and hybridization connect diverging lineages is crucial. Allopatric and sympatric speciation can be viewed as ends of a continuum defined by the amount of genetic exchange, ranging from the absence (allopatry) to substantial gene flow (sympatry). However, spatial separation and its outcome, disjunct distributions of closely

related species, might be only secondary consequences of adaptive genetic divergence under sympatric conditions, thus within a population, and not necessarily the result of passive fragmentation of populations. Recent reviews of sympatric speciation are given by Schilthuizen (2001), Via (2001), Coyne and Orr (2004), Bolnick and Fitzpatrick (2007). For a focus on theoretical models, see Gavrilets (2004).

While neither the initial theoretical demonstration of its possibility (Maynard Smith 1966, Kondrashov and Mina 1986), nor the few early case reports (*e.g.*, Bush 1969, 1975) originally had gained wide acceptance, only in recent years has the sympatric mode of speciation been reconsidered as a serious alternative based on an improved theoretical framework (Doebeli 1996, Dieckmann and Doebeli 1999, Kondrashov and Kondrashov 1999, Doebeli and Dieckmann 2003, Gavrilets 2004, Gourbiere 2004, Waga *et al.* 2007) in concert with a growing body of field evidence (Feder *et al.* 1988, Schliewen *et al.* 1994, McCune and Lovejoy 1998, Shaw *et al.* 2000, Wilson *et al.* 2000, Schliewen *et al.* 2001, Schliewen and Klee 2004, Barluenga *et al.* 2006, but see also Schliewen *et al.* 2006). However, re-evaluation of one of the famous textbook examples for sympatric speciation, the apple maggot fly *Rhagoletis*, has revealed that the picture is more complex and might also involve historical and even geographical aspects (Jiggins and Bridle 2004). Therefore, we can conclude here that sympatric speciation appears to occur in some cases, under divergent natural or sexual selection even in the presence of some gene flow. Among the most prominent factors currently discussed, particularly in the context of adaptive radiation, is ecology in concert with specialization.

Ecological speciation

An important factor involved in the formation of new species is the mechanism of initial divergence. Many evolutionary biologists, from Darwin to Mayr, were aware that ultimately speciation is also an ecological process (*e.g.*, Mayr 1947), even when later often stressing isolation as evolutionary factor (Mayr 1959). Sympatric and parapatric speciation depend on strong natural selection to work, and thus the role of adaptation has again been emphasized. Speciation involving natural selection has been termed ecological speciation by Schluter (1996). This includes but is not necessarily equal to non-allopatric speciation, with ecological specialization and both natural and sexual selection playing key roles. For a general review of the role of ecology in speciation see, *e.g.*, Orr and Smith (1998), Coyne and Orr (2004), and Butlin *et al.* (2009); for a review and discussion of the role of specialization and selection see McKinnon and Rundle (2002), McKinnon *et al.* (2004), and Irschick (2005).

Allopatric speciation is more flexible in respect to the role of selection, as random genetic processes were invoked early (peripheral isolate model, Mayr 1942) to explain the buildup of reproductive incompatibility between separated populations. Genetic processes can lead directly to reproductive isolation, *i.e.*, without natural selection being involved. Examples are genetic drift or differential acquisition of mutations in separate populations. Nevertheless, natural selection has been regarded as the major factor in allopatric speciation as well. Under this hypothesis the build-up of reproductive isolation between populations is seen as a by-product of adaptation caused by different environmental selection pressures (Mayr 1942, Dobzhansky 1951, Endler 1986).

While de-emphasizing geographical settings and re-affirming the importance of natural selection and adaptation, ecological speciation has the same appeal of intuitive appraisal as allopatric speciation. Central to this idea is to connect differences in ecologically important traits to the build-up of reproductive isolation, thus underscoring the divergence of specific traits in the absence of the homogenizing effect of gene flow between individuals. Consequently, the prediction of the ecological model of speciation is ecologically dependent isolation. In its early stage, the theory of ecological speciation was not supported by evidence, and no tests were available to distinguish it from other modes of speciation, since several aspects of ecological speciation, as for instance possible reinforcement, can occur in the late phases of about every speciation model (Schluter 2001, Coyne and Orr 2004). It should be noted that reinforcement, *i.e.*, the enhancement of (pre-) zygotic isolation in sympatry, or natural selection against maladaptive hybridization (Coyne and Orr 2004) results in rapid allopatric speciation on the one hand (*e.g.*, Hoskin *et al.* 2005), as on the other hand reinforcement by sexual selection can lead to rapid speciation under sympatric conditions, as shown for the radiation of the cricket *Laupala* on Hawaii (Shaw 2002, Shaw and Danley 2003, Mendelson and Shaw 2005). Today ecological speciation by adaptive divergence is increasingly viewed as a powerful engine of speciation, and is currently discussed as a key feature in particular of sympatric speciation.

Tests of ecological speciation include evidence for parallel evolution, *i.e.*, the development of the same phenotypic characters in closely related but independent lineages under identical extrinsic conditions (Schluter and Nagel 1995, Pigeon *et al.* 1997, McPeek and Wellborn 1998, Rundle *et al.* 2000, Mundy *et al.* 2004, Foster and Baker 2004, Colosimo *et al.* 2005), inverse correlations between morphological differentiation as an estimator of divergent selection and gene-flow as an estimator of reproductive isolation (Lu and Bernatchez 1999, Schluter 2000b), selection against hybrids (Rundle and Whitlock 2001), or assortative mating by selective environments (*e.g.*, Kondrashov and Shpak 1998, McKinnon *et al.* 2004, Seehausen *et al.* 2008). Recent models for speciation under divergent selection, comprising genetically differentiated and highly specialized populations of pea

aphids, were tested by Hawthorne and Via (2001) and Via and Hawthorne (2002). Another case study is presented in Sorenson *et al.* (2003). According to these recent views, speciation is considered a by-product of ecological differences under divergent selection on, often on only a small number of phenotypic and/or behavioral traits.

The current ecological context and natural as well as sexual selection can also be viewed as playing a decisive role in the diversification of species. Accordingly, as originally assumed implicitly by Darwin, natural selection and adaptation are the means of generating biodiversity, which is now also being discussed in context of adaptive radiations (see for reviews *e.g.*, Schluter 1996, 2000a, 2000b, 2001, 2009, Orr and Smith 1998, Sudhaus 2004, Rundle and Nosil 2005, Hendry *et al.* 2007, Patten 2008, Hendry 2009, Johannesson 2009, Nosil *et al.* 2009, Schluter 2009). Instead of geography, factors such as phenotypic plasticity, intra- and interspecific competition with character displacement and specialization as well as sexual selection lead to divergence and eventually to speciation. Thus, with ecology and selection discussed as a major player in the diversity of life, the underlying subject of allopatric versus sympatric speciation will remain a battleground in evolutionary biology for quite some time.

Hybridization and the genetics of speciation

In this context, the long debated phenomenon of interspecific hybridization, a major focus since the time of Darwin, has gained much attention lately (reviewed in Shaw and Danley 2003, Seehausen 2004, Mallet 2005, Noor and Feder 2006, Grant and Grant 2008, Schwenk *et al.* 2008, Presgraves and Yi 2009). Hybridization with introgression of alleles from one population into another is discussed as potentially enhancing adaptation, speciation and radiation, thus with potentially important consequences in evolutionary biology and speciation theory. Increasingly widespread phylogenetic evidence of cryptic introgression (even hybrid speciation at least in plants) is found in many groups, from Darwin's finches, cichlids and telmatherinid fishes to butterflies and sunflowers. An overview for hybridization is given in Coyne and Orr (2004), and recent discussion can be found in Seehausen (2004) and Mallet (2005), with case studies discussed in Glaubrecht (2010a). Using freshwater pachychilid gastropods of the genus *Brotia* H. Adams, 1866 from Thailand as examples of conflict between shell morphology and genetic diversity based on mitochondrial gene fragments (16S and COI) forming well differentiated haplotype lineages, Köhler and Deein (2010) have discussed evidence for introgression of mtDNA haplotypes, and considered hybridization between closely related species due to secondary contact the most likely cause.

Hybridization and introgression with varying levels of gene flow in the absence of strict allopatry are antagonistic to the process of speciation, but, nevertheless, might play an important role in the context of adaptive radiation (*e.g.*, Seehausen 2004, Gavrilets and Losos 2009). While estimated to occur only in 10% of animal species, mostly young species, and thus found among closely related lineages, species flocks and adaptive radiations are most likely to provide suitable targets for studies, facilitated by modern molecular methods.

Diversification and adaptive radiation

Adaptive radiation explicitly links speciation and ecology. These two widely overlapping fields of research are arguably at the core of evolutionary biology. Thus, the study of adaptive radiation has the potential to significantly influence our perception of the living world in general. Radiations represent the most spectacular examples of the outcome of speciation processes. If assemblages of numerous closely related species endemic to a geographically confined area are addressed, the term species flock is usually employed (Mayr 1963). Species flocks are outstanding for their diversity and have attracted the interest of biologists ever since their discovery. Many have achieved textbook status as examples of explosive speciation and adaptive radiation, as the Darwin finches on Galapagos (Lack 1947, Grant 1986, Grant and Grant 2008), the Hawaiian drosophilids and crickets (Zimmermann 1958, Carson and Kaneshiro 1976, Shaw and Danley 2003, Mendelson and Shaw 2005), or the cichlid fishes in the East African lakes (Fryer and Iles 1972, Barlow 2000, Salzburger and Meyer 2004, Seehausen 2006) and the anole lizards in the Caribbean (Losos 2009). However, several other less spectacular flocks exist, also among molluscs, such as *e.g.*, the partulid snails on Pacific islands (Cowie 1992, Johnson *et al.* 1993) and gastropods in so called "ancient lakes", rendering islands as well as lakes as ideal natural laboratories (Glaubrecht 2010a, Wilke *et al.* 2010). The long-lived lakes, such as Lake Tanganyika, Lake Baikal, or the Malili lakes on Sulawesi (see below) are exceptionally rich in species numbers (Brooks 1950, Coulter 1991, Martens *et al.* 1994, Fryer 1996, Rossiter and Kawanabe 2000, Glaubrecht 2008a, Glaubrecht and Rintelen 2008a, Rintelen *et al.* 2010).

All these species flocks are regarded as examples of adaptive radiation, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage, thus linking speciation and ecology (Schluter 1996, 2000a, 2000b, Glor 2010, Losos and Mahler 2010). Accordingly, three main processes are involved in radiations: (i) phenotypic divergence of natural populations driven by natural selection between environments, (ii) phenotypic divergence mediated by competition for resources, and (iii) ecological speciation. While some of these processes have been challenged, *e.g.*, competition and associated character displacement or the role of key innovations (Seehausen 2006), the concept of adaptive radiation has enjoyed wide recognition and interest

since the establishment of the modern synthesis. Within this framework, extrinsic factors such as major geographic changes provide the opportunity, and intrinsic factors such as key innovations in concert with adaptation and specialization provide the potential for radiation (Simpson 1953, Mayr 1963, Liem 1990, Schluter 2000b, Losos and Mahler 2010, but again see Seehausen 2006).

In conclusion we can say that the central controversy over models in speciation is far from being settled, as it is not yet resolved what genetic change will result in a new species and under which conditions, thus what the actual influence on speciation is of genetics, geography and adaptation to the environment. However, with new genetic research and genomic techniques homing in on the molecular and cellular mechanisms that enable diversification to occur, our current understanding of the speciation process and adaptive radiation has improved considerably. Eventually, this will provide answers to the next generation of questions on the frequency and importance of different processes that cause speciation.

MOLLUSCS IN SPECIATION STUDIES

"The most memorable lesson I learned from Darwin is that the most important thing in scientific research is not *to add to the accumulation of facts, but to ask challenging questions about the how? and why? of biological phenomena and to try to answer them."* Mayr (1999: 403)

The way in which speciation proceeds can only be fully understood by analyzing data collected from a large number of different model systems. To date, essentially only vertebrates are used (mostly birds and fishes, see above), while speciation mechanisms remain widely untested for many invertebrates, with the notable exception among insects, of butterflies, diptera and crickets. As evolutionary biologists working with molluscs, we should aim at testing the universality of known and disputed speciation mechanisms, and it is with a clear focus on these mechanisms we should choose our molluscan models to increase their frequency as a source of data in order to decipher the underlying mechanisms of biodiversity.

As was shown by Schwenk *et al.* (2008), mollusc species hold the same chance for studying evolutionary phenomena, in this case hybridization, as other groups. However, as noted before, *e.g.*, in case of the term "adaptive radiation" (Glaubrecht 2008b), too often malacologists use evolutionary biology issues for the titles of their papers or book section only, in lieu of a soundly designed study. As much as the term "adaptive radiation" is often misused, "speciation" not only reflects the taxonomic description of any speciose group, but instead the actual study of causation and underlying mechanisms of how species arise. Consequently, we should not merely focus on the final stages of this process revealed in a fixed pattern, but actually attempt to study the complete process leading to a particular species assemblage. One symptomatic example for the ill-informed mis-use of the term "speciation" is Bandel's (2000) typological catalogue of chronospecies (at best, if at all distinct evolutionary entities) among the highly polymorphic fossil freshwater Melanopsidae. For a discussion of the over-naming of melanopsid forms and fossils see Glaubrecht (1993, 1996). Suffice it to state, as only one more example, Lazarus's (1983, 1995) attempts to deduce a sympatric mode of speciation from the microfossil record of pelagic protista such as foraminifera; other examples are discussed in Benton and Pearson (2001). From the debate among neontologists as to the paramount difficulties of distinguishing this particular mechanism from others (see above) we should at least have learned to meet such claims with much more reservation.

Instead of referring to "speciation" or "adaptive radiation" for each and every speciose taxonomic group we should strive to investigate, with adequate methods and founded on solid theoretical ground, the underlying mechanisms of anagenetic versus cladogenetic change. Then, certainly, molluscs can contribute to studying the highly interesting, yet controversial speciation mechanisms, hybridization and adaptive radiation. Not only freshwater snails (see below) and land snails (*e.g.*, Gittenberger 1988, Jordaens *et al.* 2009, to mention only very few here) but also marine gastropods have figured prominently in these investigations (Allmon and Smith 2011).

We still lack textbook examples of particular molluscs for sympatric and ecological speciation, adaptive divergence, and hybridization that are equally widely known let alone well studied, as provided among the vertebrates. Yet, there are suitable candidates among gastropods in ancient lakes, from oceanic islands and archipelagos, mountains or other insular environments with endemic species, as is evident from the contributions to a recent symposium on speciation in such environments (Glaubrecht 2010b).

Marine mollusc studies

For aquatic animals, in particular molluscs, the marine and limnic realm and their organisms are studied separately. For example, Bilton *et al.* (2001) and Bohonak and Jenkins (2003) discussed evolutionary ecological implications of speciation and dispersal in freshwater organisms, while Palumbi (1992, 1994, 1996) reviewed similar aspects including genetic divergence and reproductive isolation for marine organisms. In addition, the many detailed case studies on speciation mechanisms and on historical biogeography only look at one aquatic environment, largely ignoring taxa with amphidromous life history and habits that provide most elegant models for testing known hypotheses on speciation,

species diversity and range extension; for Cerithioidean gastropods see Glaubrecht (1996).

Recently, molecular genetic studies on various marine taxa have shown that the World's oceans are in fact not homogeneous areas as commonly held and that populations are much more structured in terms of their genetics than previously assumed. In many cases species ranges coincide with biogeographical boundaries as was shown for various gastropod taxa (*e.g.*, Hellberg 1998, Collin 2001, 2003, Williams *et al.* 2003, Williams and Reid 2004, Johannesson and André 2006, Reid *et al.* 2006, 2008, 2010, Lind *et al.* 2007, Duda *et al.* 2008, Malaquias and Reid 2009). To evaluate the various explanations, such as isolation by distance or stepping stone dispersal, recent studies compared biogeographical patterns and infered a specific (in this case allopatric) mode of speciation in concert with vicariance, due to plate tectonics and in light of age estimates of the events (Williams and Reid 2004, Williamson and Duda 2008, Reid and Williams 2010). However, for most marine taxa the phylogenies have not been established sufficiently to allow detailed investigation on range expansion, with the aim of looking into correlation of potentially narrow range occurrences and speciation.

Hinting at the "marine paradox", *i.e.*, that marine species often have pelagic larvae and extensive geographical ranges in a seemingly homogeneous ocean, suppressing allopatric speciation, Allmon and Smith (2011) reviewed examples of speciation in (fossil) marine molluscs. Although it is expected to find an allopatric pattern and mechanism (see above, for a contrary view *e.g.*, Johannesson 2009), allopatry can have quite different causation, such as vicariance by tectonics or climatic and oceanographic factors as well as by dispersal across still existing geographic barriers. Sophisticated molecular phylogenetic studies and phylogeographic analyses are needed here for an evaluation of geological age and the exact mechanism of marine speciation. For more discussion on the phylogenetic approach, see below.

While the plethora of studies in this context essentially use phylogenetic and phylogeographic data to infer speciation mechanisms, only in very few cases are detailed analyses performed looking into the details of the causation for speciation. One example comes from a series of papers on the strongly polymorphic marine snail *Littorina saxatilis* (Olivi, 1792) studied by Johannesson (*e.g.*, 2001, 2009, see also Johannesson *et al.* 1995, Wilding *et al.* 2001, Johannesson and André 2006, Quesada *et al.* 2007) using parallels in populations of sympatric morphs and/or ecotypes with overlapping distributions and hybrids to suggest habitat selection, non-random (*i.e.*, assortative) mating and possibly sexual selection to not only contribute to, but maybe even cause sympatric reproductive isolation. However, we should not mistake such cases of parallel speciation as indicating only sympatric divergence and sympatric speciation, as suggested by Johannesson (2001, 2009). Parallel speciation also occurs between allopatric populations and ecotypes that could once have arisen in allopatry and only later became sympatric (Schluter *et al.* 2001).

Freshwater molluscs

With respect to freshwater gastropods we are even further away from really understanding the details of species formation that leads to the array of radiations described for many taxa and cases. Again, speciation should not be understood as merely reflecting the compilation of species descriptions of any speciose group, as I have criticized above. More importantly, there are two main problems involved here: First, the difficulty still remains on the species level that molecular techniques in concert with lineage-based thinking have lead to an increase (by an estimated 20 to 60%) in the number of what is called in these studies either "phylogenetic species" or "evolutionary significant units", often applied in avoiding to assess the status as distinct (bio-) species. This problem of overnaming, or taxonomic redundancy that in the past has caused the existing plethora of synonyms, in concert with the increased assignment of insufficiently differentiated subspecific populations to ("cryptic") species status, both lead to a taxonomic inflation that needs much more attention, as pointed out in Glaubrecht (2004, 2009). Glaubrecht *et al.* (2009) provide a case study among Australian freshwater thiarid gastropods. Only now, after more than a decade of the unhealthy spread of DNA barcoding, this approach is rightly criticized as being an anti-intellectual endeveour (see *e.g.*, reviews in Lee 2004, Meier 2008, Ebachs and Carvalho 2010).

Second, speciation is not to be understood as being revealed comprehensively by particular phylogenetic patterns since it is the result of a more complicated and continuous process. We need to realize more often that the cladograms, or "cloudograms" (as they have been more properly termed, Zachos 2009) resulting from sequence analyses of partial fragments of very few genes are far from being the best way to truly reflect details of the process of formation of species. Accordingly, these patterns only allow very limited inferences on the speciation process involved.

Thus, it is not surprising that we see a plethora of pattern-based studies with statements on lineage-base "species", but only few reliable insights into the underlying causation of speciation itself. Even for most speciose freshwater taxa, such as *e.g.*, the rissooidean *Bythinella* Moquin-Tandon, 1856 with wide species distribution in continental Europe (Bichain *et al.* 2007, Haase *et al.* 2007, Benke *et al.* 2009, Wilke *et al.* 2010), or hydrobiids in North America (Hershler *et al.* 2005, Hershler and Liu 2008), essentially only phylogenetic approaches and phylogeographical analyses are available. Most authors

struggle with the paramount problem of species delimitation without further exploring speciation mechanisms. This holds true, for example, for freshwater gastropods such as the Ancylidae (*e.g.*, Pfenninger *et al.* 2003, Walther *et al.* 2006) and Neritidae (Feher *et al.* 2009), with the genetic, ecological and morphological differentiation studied, or the Viviparidae (Sengupta *et al.* 2009) and Ampullariidae (Haynes *et al.* 2009a, 2009b), with a focus again on aspects of global phylogeny and biodiversity. More examples, in particular among the lacustrine and riverine freshwater Cerithioidea, will be reviewed and discussed below.

Unfortunately, not only the fossil record (see below) but also standard molecular phylogenetic analyses (in particular when using mtDNA gene fragments such as the bar coding gene COI) often lack the temporal and spatial resolution to allow any more detailed inferences on speciation modes. Being either explicitly or implicitly aware of this, only very few studies claimed evidence for incipient speciation in aquatic snails, albeit deduced merely from a gene tree (*e.g.*, Colgan and Ponder 2000). Other studies decisively ruled out the otherwise often implicitly suggested hypothesis of so-called "cryptic speciation" when finding large COI divergences (*e.g.*, Walther *et al.* 2006). However, to date none of these studies (with the exception of those in "natural laboratories", see below) are experimentally designed and/or conducted to actually allow for detailed insights into how exactly species multiply in molluscs.

One of the urgent questions to answer is, for example, how far different modes of speciation contribute to the diversity among and within particular taxa. Deduced from a given phylogenetic pattern more often than not an allopatric setting is assumed, while a discussion of other, *e.g.*, ecological factors, is largely lacking. In this context, an integrative evolutionary ecology approach has been suggested, aiming to reconstruct the origin and alteration of the ecological interrelations of organisms and their respective environments in the course of evolution, as defined and explained in more detail by Glaubrecht (1996). This approach provides a research program for acquiring a synthetical perspective that includes morphology, molecular genetics, ecology and biogeography, in order to look into the speciation process beyond the point where we simply assume geographical factors (allopatry). Bilton *et al.* (2001) and Bohonak and Jenkins (2003) reviewed some of the evolutionary ecology implications in freshwater organisms in general, while Glaubrecht (1996, 2000) has discussed these for limnic groups among the Cerithioidea.

One central aspect in this context is, of course, the question of dispersal and colonization ability, thus gene flow and genetic divergence, of taxa that are highly restricted by their essentially patchy habitat. Since active as well as passive dispersion can rarely be investigated directly, inferences have to be made via mostly molecular markers. Depending on the taxon and occurrence, the habitat conditions, *e.g.*, drainage system, lacustrine constellation, and geographic isolation, have to be considered in concert with intrinsic biological properties. However, these are only rarely studied (Bilton *et al.* 2001). The review of Bohonak and Jenkins (2003) discloses how far away we are from a thorough understanding of the evolutionary ecology of freshwater gastropods, truly integrating knowledge on dispersal, gene flow, life history into a phylogenetic and phylogeographic framework. As we lack any more detailed studies as well as verification and general theory on this, some authors (Harvey 2002, Ponder and Colgan 2002) have simply claimed that among freshwater molluscs so-called "narrow range" taxa (*i.e.*, those with restricted occurrences) tend to speciate more. Similar claims that, for example, viviparous taxa among limnic gastropods are generally not only more restricted as to their distribution but also, as a rule, more speciation-prone (*e.g.*, Michel *et al.* 1992, 2004, Michel 1994, 2000) have been shown to be essentially unsupported by evidence (see, *e.g.*, discussion in Glaubrecht 1996, 1999, 2006).

How much malacology contributes to important issues in evolutionary biology and speciation in particular can be seen from the review and papers given during the World Congress of Malacology 2007 in Antwerp, and its resulting publications (see Glaubrecht and Rintelen 2009 and references therein), as well as from the present volume (Allmon and Smith 2011). Further details will be given for those cases which I discuss below for studies on limnic lineages among the Cerithioidea (Fig. 1).

PATTERN AND PROCESS OF SPECIATION IN CERITHIOIDEA: EMPIRICAL APPROACHES

"One lesson to consider is that when theoretical frameworks are lost, empirical information disappears with them." Nyhart (2002: 12)

Unfortunately, malacologists felt that describing any assemblage of species (names) of living or fossil taxa as well as analyzing alignments of partial gene fragments with standard algorithms already justifies the attribute of any kind of speciation study. With respect to our "Darwinian mysteries", or the big questions for the near future of biological sciences (see Glaubrecht 2009), though, the subject of speciation is more complicated than reflected in these simplistic approaches.

First, we need to realise one misleading aspect of supposedly "modern" biodiversity research. Our yet unresolved questions concerning species numbers, species concepts and speciation go well beyond those aspects covered by the many current biodiversity inventory projects. Sadly, these are

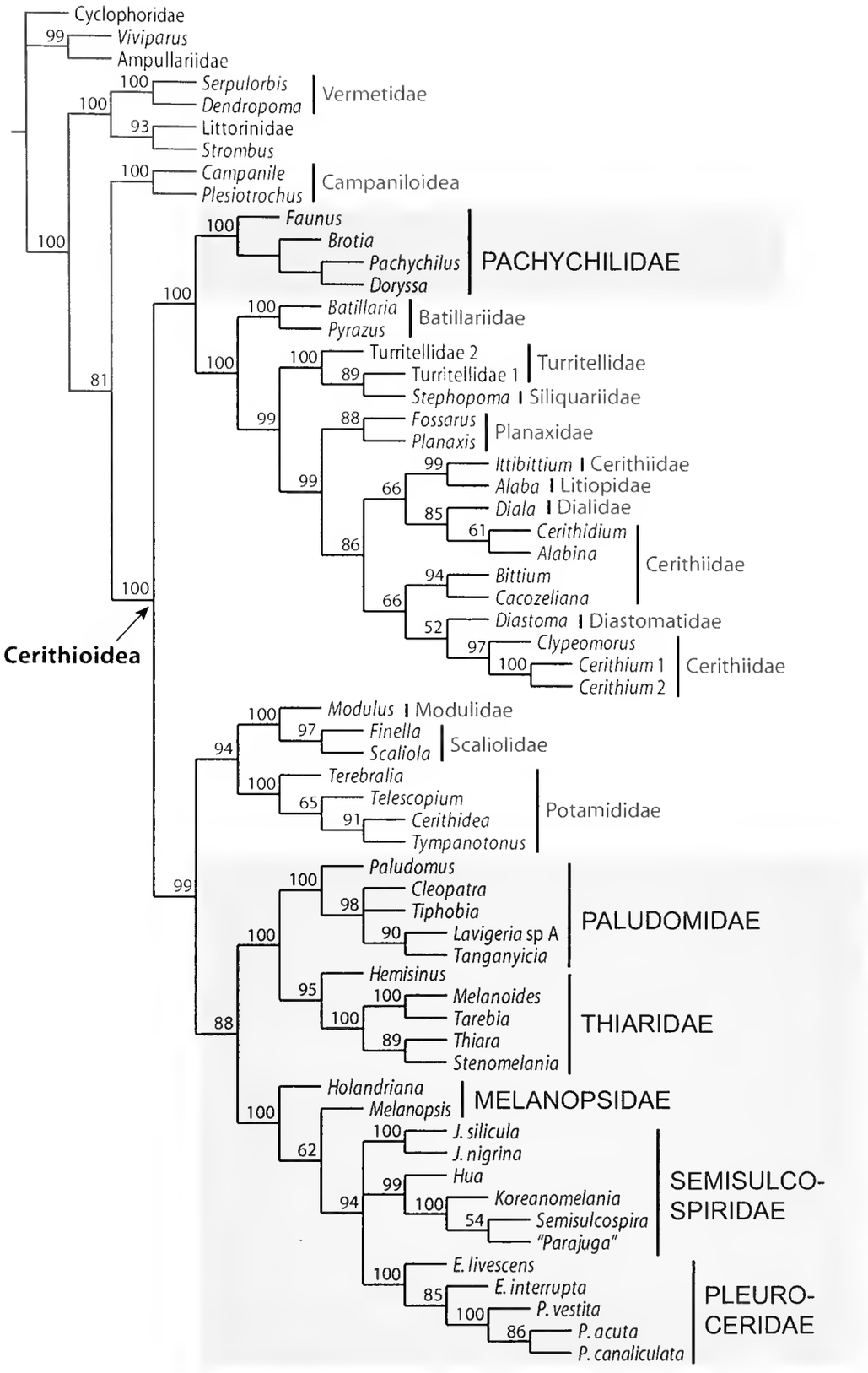

Figure 1. Cladogram of the caenogastropod superfamily Cerithioidea, based on the Bayesian analysis of a combined morphological and molecular dataset (16S, 28S, with unconserved regions removed), modified from Strong *et al.* (2011). Freshwater lineages are highlighted.

limited to simply cataloging and inventoring all given names or taxa of an area, rather than attempting to face the paramount problems with species definitions and delimitations, synonymy and taxonomy redundancy, or any understanding of forces that ultimately result in the patterns found. We yet have to resolve, for example, whether species richness is a reliable proxy of (accelerated) speciation and whether any species assemblage or flock is a proxy of any particular mechanism of speciation.

Second, as we have seen above, inferences from geographic patterns have dominated the literature, together with the study of allopatric speciation (*e.g.*, Mayr 1942, 1963). Many molecular phylogenies and in particular phylogeographic studies are now available (Hickerson *et al.* 2010); for aquatic molluscs see Wares and Turner (2003). However, our interpretation of phylogenetic as well as biogeographic patterns largely depends on our concepts of the modes of speciation and cladogenesis in general, as was rightly pointed out by Heads (2009), although he only discussed dispersal versus vicariance, and a new method of analyzing the biogeography of a given group. While the statistical rigor of the new discipline of phylogeography has increased, and an ambitious synthesis is envisioned (Hickerson *et al.* 2010), we still lack deeper insights into the mechanisms of speciation. While Barraclough and Vogler (2000) have suggested how to investigate the role of speciation from species-level phylogenies, Losos and Glor (2003) remained sceptical and have even proposed that interspecific phylogenies are unable to test alternative hypotheses of speciation, for example the role of geology and the adaptive process.

Irrespective of this criticism and discussion, we currently employ three empirical approaches in the study of speciation:

(i) the phylogenetic comparative method, *i.e.*, looking into pattern of molecular phylogenies,
(ii) fossils, *i.e.*, inferring the history of a clade through time, and
(iii) microevolutionary studies of extant taxa, with focussing on traits and processes.

Contrasting lacustrine with riverine settings, I discuss these issues here exemplified by our studies on several pantropically distributed limnic Cerithioidean gastropod taxa (Fig. 1), which range from the New World and the Mediterranean region to lakes and rivers in East Africa and SE Asia to Australia. A taxon-by-region overview of the six distinct phylogenetic lineages of freshwater Cerithioidea currently considered on the family level is presented in Table 1. It should be noted that these six families were traditionally considered as "Melaniidae" until only a few decades ago, and that several of these distinct lineages were then for long subsumed under Thiaridae. However, our phylogenetic analyses of constituent groups as well as within the Cerithioidea context (reviewed in Glaubrecht 1996, 1999, 2006, Lydeard *et al.* 2002, Strong *et al.* 2011) have revealed the existence of two (or potentially three) independent lineages that probably colonized freshwater since the Mesozoic (Fig. 1).

It is in the context of speciation and radiation that these freshwater Cerithioidea have recently been described as truly "Darwinian snails", based on studies on paludomid snails in East Africa's Lake Tanganyika (Glaubrecht 2008a), the freshwater pachychilid *Tylomelania* Sarasin and Sarasin, 1897 endemic to rivers and central lakes on the Indonesian island of Sulawesi (Glaubrecht and Rintelen 2008a, Rintelen *et al.* 2010), two endemic riverine *Pseudopotamis* Martens, 1894 on the Torres Strait Islands (Glaubrecht and Rintelen 2003), *Brotia* in rivers in central Thailand (Glaubrecht and Köhler 2004, Köhler *et al.* 2010), and the riverine *Madagasikara* Köhler and Glaubrecht, 2010 on Madagascar (Köhler and Glaubrecht 2010).

Phylogenetic comparative method: patterns of molecular phylogenies

With an increasing armamentarium of molecular genetic techniques for exploring the genetic structure of populations, species and higher level taxa, the one mystery of how species originate has become many, and possible solutions multiplied. Not only have we uncovered further complexities in what we mean by species, but we also gained further insights into the drivers and rate of speciation. However, molecular systematists realize now that cladograms are actually more like "cloudograms". Gene fragments reflect gene genealogies, or molecular "gene trees", but are not identical to organismic phylogenies, or "species trees", a fact that was pointed out early by Maddison (1997). The lack of identity is explained, among other causes, as due to the coalescent process of species divergence, resulting over time from genetic drift and incomplete lineage sorting (*i.e.*, the maintenance of an ancestral allele polymorphism across population or species boundaries with the coalescence process not completed for

Table 1. The biogeography of freshwater Cerithioidea taxa, listed by family, on a global scale. The six families, with their constituent taxa, as currently conceived, are given here as delimited in Glaubrecht (1996, 1999, 2006), Glaubrecht and Rintelen (2008b), and the most recent overall phylogenetic analysis in Strong *et al.* (2011). For species-level classification see references listed in the footnotes.

Region	America / Caribbean	Europe / Palaeartic	Africa	Asia	Australia / NZ
taxon	Pachychilidae		Pachychilidae[1]	Pachychilidae[2]	Pachychilidae[3]
	Pleuroceridae[4]				
	Semisulcospiridae[5]			Semisulcospiridae[6]	
		Melanopsidae[7]			Melanopsidae[8]
			Paludomidae[9]	Paludomidae	
	Thiaridae[10]		Thiaridae[9]	Thiaridae[11]	Thiaridae[12]

[1] With *Potadoma* on continental Africa and *Madagasikara* endemic to Madagascar, see Köhler and Glaubrecht (2010)
[2] Köhler and Glaubrecht (2001, 2003, 2006, 2007), Köhler and Dames (2009), Rintelen *et al.* (2007)
[3] With only two species of *Pseudopotamis* endemic to the Torres Strait Islands, see Glaubrecht and Rintelen (2003)
[4] Restricted to North America, for phylogeneric analysis see Holznagel and Lydeard (2000) and Strong and Köhler (2009)
[5] Restricted with *Juga* to the western drainages in North America, see Holznagel and Lydeard (2000) and Strong and Köhler (2009)
[6] Restricted to Far East Russia, Japan, and Korea, for phylogeneric analysis see Strong and Köhler (2009)
[7] Glaubrecht (1993, 1996)
[8] With few melanopsid species restricted to New Caledonia and New Zealand only, see Glaubrecht (1996)
[9] Wilson *et al.* (2004), Glaubrecht (2008a), Glaubrecht and Rintelen (2008b)
[10] With Hemisininae on Caribbean islands and mainland South America only, Glaubrecht and Rintelen (2008b)
[11] Glaubrecht (1996, 1999, 2006), Glaubrecht and Rintelen (2008b)
[12] Glaubrecht *et al.* (2009)

the respective gene), or from hybridization. In addition, molecular geneticists have repeatedly discussed necessary optimization and shortcomings of mtDNA sequence data in phylogeography and molecular ecology (Avise 2000, 2006, Whelan and Goldman 2001, Funk and Omland 2003, Lee 2004, Meier 2008). Not only is species-level paraphyly and polyphyly far more common (up to 23% of studied taxa) than generally recognized, calling for careful interpretation of species limits and speciation phenomena, but the ignorance of the discordance of gene and species trees is widespread. Many authors take the results of their single-locus studies at face value, as discussed in Zachos (2009), producing highly unsatisfying taxonomies.

Accordingly, phenomena such as coalescence and processes such as incomplete lineage sorting need to be taken into account in systematics and evolutionary biology to avoid erroneous conclusions. We need to realize that gene trees resulting from many of our recent (phylo) genetic studies are at best "weak null hypotheses of population relationships" (Dillon, pers. comm., 2008), allowing only poor inference regarding the actual evolutionary relationship between species. I will hint at only a few examples where this holds true in freshwater gastropods (*e.g.*, Walther *et al.* 2006, Sengupta *et al.* 2009) and among the Cerithioidea in Pachychilidae (Rintelen and Glaubrecht 2002, Glaubrecht and Köhler 2004, Köhler and Glaubrecht 2006, Köhler and Dames 2009, Köhler and Deein 2010, Köhler *et al.* 2010, Rintelen *et al.* 2010) and in Pleuroceridae (*e.g.*, Dillon and Frankis 2004, Lee *et al.* 2007, Dillon and Robinson 2009).

Nevertheless, molecular species-level phylogenies and the comparative method still allow certain insights into the origin of diversity by speciation and radiation, albeit mostly without explicit inference of the exact mechanisms. For example, two of our own studies on brackish-water Cerithioidea revealed interesting patterns as to the presence of more speciose clades among species-poor or even monotypic genera within the Potamididae (Reid *et al.* 2008) and the Batillariidae (Ozawa *et al.* 2009), both common in the Indo West-Pacific. In potamidids we correlated the origin of the 29 living species with a speciation event in the Tethyan realm in the Middle Eocene, and the appearance of mangrove habitats on which they still depend today, thus suggesting that potamidids in fact do represent an adaptive radiation and have always been closely associated with mangroves. We also found the 14 living batillariid species to represent a Tethyan relict, reaching Australasia by the Late Oligocene, with four genera still surviving in this refugium. Only within one lineage, *Batillaria* Benson, 1842 that started to migrate north in the Early Miocene, our phylogenetic analyses revealed a radiation into eight species. The remaining genera are species-poor if not even monotypic.

Of course, those patterns are interesting in themselves as they help to establish the phylogenetic framework. Applying phylogenetic methods yields patterns of genetic affinity that allow us to judge past events of divergence. Nevertheless, the comparative method only provides a first indication for speciation under the influence of either geographical or ecological factors, pointing out future avenues towards a truly evolutionary ecology approach. We are far away from this goal with respect to the phylogeny and phylogeography of freshwater cerithioidean families such as the Pachychilidae (Köhler and Dames 2009, Köhler and Glaubrecht 2010), the Paludomidae (Wilson *et al.* 2004), Semisulcospiridae (Strong and Köhler 2009), the Melanopsidae and Thiaridae (Glaubrecht and Rintelen, unpubl. data) as well as the Pleuroceridae (Glaubrecht and Köhler, unpubl. data).

Fossils: inferring history of clades through time—case study from Lake Turkana

"Paleontology is the historical science that weaves historical narratives around evolving species." Hull (1989: 186)

The fossil record provides not only a wonderful but the only direct documentation of events in the history of evolution. The discipline has seen a revolution over the last few decades (Sepkoski and Ruse 2009), and speciation has been at the forefront of modern paleontological theory since Eldredge and Gould (1972) first proposed their theory of "punctuated equilibrium", a model for discontinuous tempos of change in the process of speciation and the deployment of species in geological time. Since then, improved approaches to speciation in the fossil record have been claimed (*e.g.*, Erwin and Anstey 1995) and speciation modes and case studies reviewed (*e.g.*, Allmon 1992, Gould and Eldredge 1993, Benton and Pearson 2001, Hendry 2008, Allmon and Smith 2011). In this context, stratigraphic resolution and acuity are of paramount importance. As high-resolution, stratigraphic sequences with appropriate time controls are generally rare or lacking in most formations and fossil strata, confirmation of speciation has been difficult to find in fossils. Nevertheless, in rare cases it is possible to infer evolutionary processes, including even adaptation by natural selection and the formation of new speciation from temporal patterns in phenotypic traits, such as size, shape or sculpture in molluscs. These patterns are, for example, consistent directional trends, constancy (or stasis) and randomness. See Hendry (2008) for discussion of an examplary fossil sequence in sticklebacks from an ancient lake.

Undoubtedly, for the fossil record of freshwater gastropods, Williamson's (1981, 1985) claim of having found evidence for speciation and punctuated equilibrium in Plio-Pleistocene molluscs of the East African Turkana formation is still the most famous, albeit also highly controversial one. Next to bivalves (which are not considered here), the

most abundant fossil gastropods found in the Lake Turkana beds spanning 2 my belong to the two Cerithioidean families Paludomidae (*Cleopatra* Troschel, 1857) and Thiaridae (*Melanoides* Olivier, 1804). Starting with Williamson until recently these were confused as both being thiarids with the same biological properties, such as *e.g.*, reproductive mode. However, the widely distributed paludomids in Africa are oviparous and gonochoristic (with exceptions discussed in Glaubrecht 2008a, 2010c, Glaubrecht and Strong 2007), while all thiarids are viviparous with a sub-hemocoelic brood pouch and, additionally, with *Melanoides* also being parthenogenetic (Glaubrecht 1996). In addition, to control for stratigraphy, these differences in life history of the Turkana molluscs are crucial for the interpretation in context with the claim of speciation in general and of evolutionary modes involved in particular.

Williamson (1981, 1985) described peculiar morphological changes in the "iconic" gastropods, attributing it to radiation under the punctuated equilibrium theory and (sympatric) speciation pulses followed by extinction of the, curiously enough, new species. Critics doubted this change being evidence for speciation and radiation in the fossil record, and alternatively suggested he merely documented ecophenotypical responses to environmental changes under stress. For a review of the debate see Williamson (1985) and Glaubrecht (1996: 140-142, 373-378, and references therein).

It is important to take into account that, of all thiarids known, in particular *Melanoides tuberculata* O. F. Müller, 1774 is phenotypically highly plastic in several shell traits. Nevertheless, changes in shell morphology in this and other molluscs can indeed be correlated to facies changes which we re-considered within a revised stratigraphic framework. Our own ongoing study of the Turkana molluscs revealed that they actually provide one of the rare cases of high-resolution stratigraphic sequences with appropriate time controls. Re-studying the time sequence of *Melanoides tuberculata* and *Cleopatra ferruginea* I. Lea and H. C. Lea, 1850 from the original material in the Williamson Collection, Museum of Comparative Zoology at the Harvard University, as well as our own collections done in 2006 and 2010 at Turkana, allowed us to reconstruct faunal successions instead of speciation. For preliminary results see Scholz and Glaubrecht (2007), Glaubrecht *et al.* (2009) and Scholz *et al.* (2009); a detailed account will be published elsewhere. As illustrated in Fig. 2 for one exemplary part of the Turkana record, normal-sized and intermediate *Melanoides* morphs are found to succeed each other, later being replaced by small, needle-like morphs. These latter phenotypes resemble salinity-stressed recent *M. tuberculata* living in coastal lagoons and brackish creeks (Glaubrecht, unpubl. data).

In contrast to Williamson's earlier hypothesis, but in concurrence with recent stratigraphic and sedimentological studies (Craig Feibel and Henning Scholz, unpubl. data), morphological change in *Melanoides* has to be viewed as being gradual, while only few abrupt changes are associated with pronounced alterations in facies or sedimentological interruptions. Accordingly, we propose that fluctuating environments in the region of the Turkana paleo-lakes triggered the observed morphological variation in gastropod shells, leading to the repeated extinction of lacustrine populations and later immigration of riverine populations into paleo Lake Turkana, mimicking punctuated equilibrium with stasis and sudden bursts of radiation (Scholz and Glaubrecht 2007, Glaubrecht *et al.* 2009, Scholz *et al.* 2009). Although we found support for Williamson's original description of highly resolved morphological change through time, his interpretation of rapid bursts of (punctuated) evolutionary change has to be replaced by the alternative that these changes documented in the fossil record resulted from the invasion of extra-basinal, con-specific populations. Therefore, the Turkana gastropods (and most likely other molluscs as well) do not provide a case study for the theory of punctuated equilibrium and no documentation of speciation events in fossils.

Microevolutionary studies of extant taxa: focussing on traits and processes

"If there is the slightest foundation for these remarks, the zoology of Archipelagos will be well worth examining; for such facts [would] undermine the [theory of the] stability of Species." Darwin 1836 (in Barlow 1963: 201)

While speciation occurs faster than generally observable in the (punctuated) fossil record, evolutionary events are considered too slow for study on biological timescales. Long before Georg Evelyn Hutinchson (1965) hinted at the ongoing "*evolutionary play*" in the "*ecological theatre*" of lakes as microcosms, Charles Darwin's son, Francis Darwin (1909: xii, 26) first mentioned the Galapagos islands "*as a microcosm of evolution*". In this tradition and given our focus on speciation and radiation, we consider oceanic islands and inland lakes as equally well-suited microcosms for exemplary microevolutionary studies. The rise of molecular genetics and its rapid application in phylogenetic systematics has produced over the last two decades new phylogenies for classical cases of adaptive radiation, such as the Galapagos finches, but also phylogenies for new model systems, such as the cichlids of lakes in the Neotropics and Africa (see above) as well as our limnic molluscs.

Much has been written on species flocks in particular in "ancient" or long-lived lakes, which have featured prominently in evolutionary biology, often regarding these evolutionary theatres also as hotspots of diversification, as they are exceptionally rich in species. As well as their fish radiations, ancient lakes such as Lake Tanganyika, Lake Malawi, Lake Baikal and Lake Biwa also harbor one or more

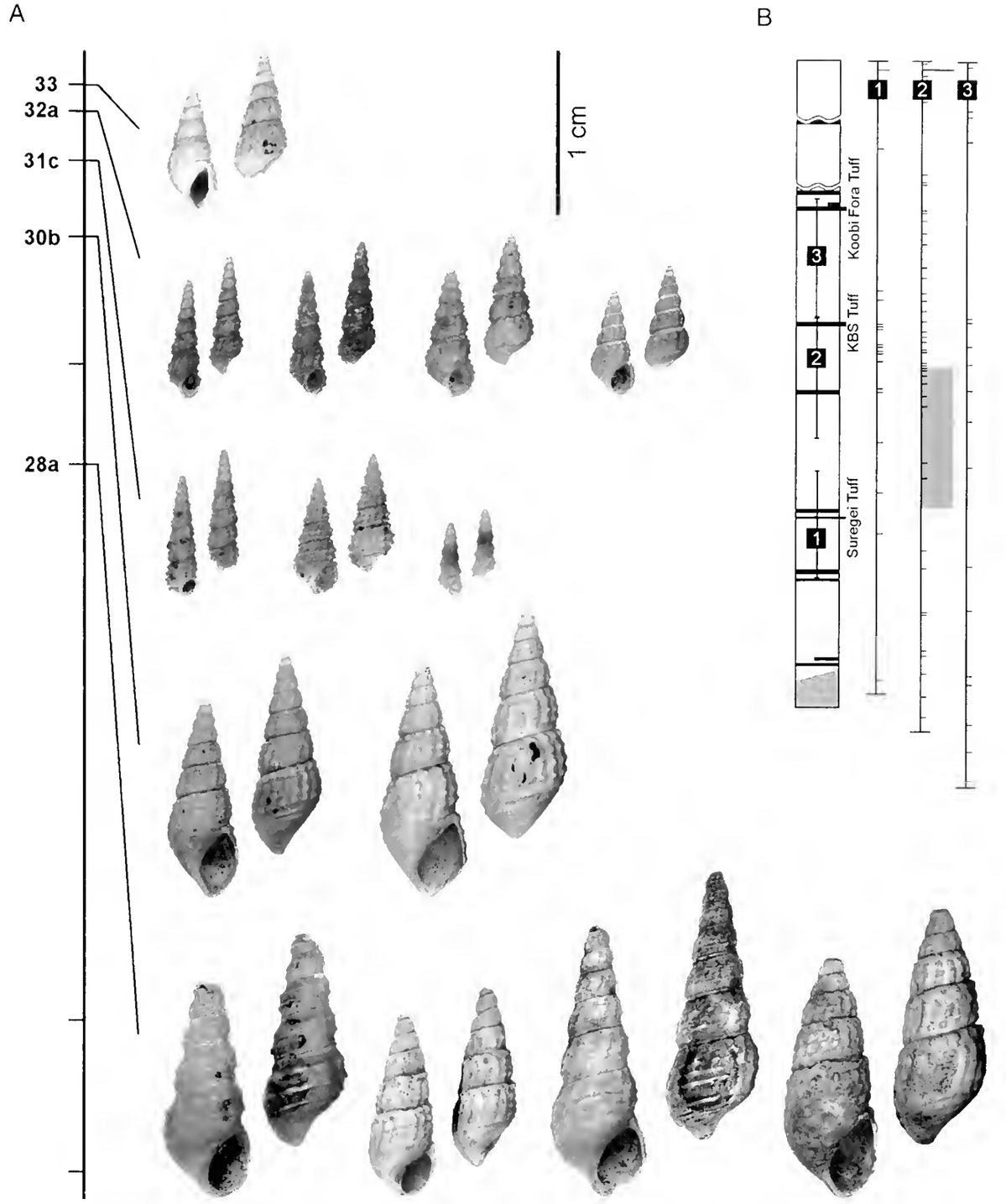

Figure 2. A, Reconstruction of a partial section of the faunal succession in *Melanoides tuberculata*, a thiarid gastropod from the Plio-Pleistocene Koobi Fora Formation in the eastern Turkana Basin of East Africa. Typical representatives of mollusc faunas from the original Williamson Collection, numbered from 28a to 33. Note the appearence of needle-like forms from fauna 31c onwards, with the morphological variability being higher and shell size much smaller than in older and younger strata. B, The composite stratigraphic column and divisions of the Turkana Basin deposits with stratigraphical distribution of the mollusc faunas, modified from Williamson (1981). The shaded area marks the partial section shown in A.

invertebrate species flocks of gastropods, bivalves, crabs and shrimps (examples and reviews *e.g.*, in Brooks 1950, Boss 1978, Coulter 1991, Fryer 1991, 1996, Martens 1994, Rossiter and Kawanabe 2000, Wilke *et al.* 2008, Glaubrecht 2010). Derived with modern methods, new molecular phylogenies have challenged older ideas about the age and origin of these

species flocks, while offering opportunities to rigorously test hypotheses on modes of speciation, divergence times, character evolution and adaptation.

I focus on the gastropods from two of these ancient lakes as well as some rivers, since both settings provide ideal model systems to study speciation processes with respect to morphological, molecular genetic, geographical and ecological aspects. In particular, the (presumptive) endemic evolution of a gastropod species flock in the East African Lake Tanganyika, as well as that in central lakes on Sulawesi, Indonesia, provide instructive comparative case studies of speciation and radiation. In contrast, riverine radiations are both rarely known and less well studied. Although making up less than 1% of the world's water, running freshwater, or lotic systems, are more permanent on both ecological and evolutionary timescales than most lakes, or lentic habitats. Thus, fluviatile radiations make an interesting contrast to known lacustrine radiations, since they allow us to judge the decisive role of the environment (riverine versus lacustrine setting) in speciation modes, *i.e.*, allopatric versus ecological speciation (Table 2).

Species flocks in ancient lakes—Cerithioidean gastropods as models

Irrespective of being suitable model systems, our knowledge on the taxonomy, systematics and evolution of several snail species flocks in ancient lakes remained rudimentary for long. Members of these radiations possess unique morphologies very different from those of their riverine relatives, and display a wide array of phenotypic and ecological diversity, as illustrated by Lake Tanganyika gastropods (Glaubrecht 2008a) and the Sulawesi lakes (Glaubrecht and Rintelen 2008). Interestingly, as a rule no riverine species are found in these lakes and *vice versa*. Two hypotheses have been put forward to explain the different shell morphologies of lake and riverine species: escalation

Table 2. Evaluation of speciation and adaptive radiation in freshwater Cerithioidean gastropods from lacustrine and riverine settings, applying the four formal criteria suggested by Schluter (2000a). For details and explanation see text. +, verification by the study cited below; -, lack of this criterion; ?, lack of respective data.

	Phylogenetic criteria:		*Ecological criteria:*	
	Common ancestry	Rapid speciation	Phenotype-environment correlation	Trait utility
Lacustrine setting:				
- Paludomidae	+/+[1]	+[2]	?	?
thalassoid species, Lake Tanganyika	(Wilson *et al.* 2004, Glaubrecht and Strong 2007)			
- Pachychilidae	+[3]	+	+	?
Tylomelania, central Lakes on Sulawesi	(Rintelen *et al.* 2004, 2007, 2010)			
Riverine setting:				
- Paludomidae	+	?	?	?
Potadomoides, Congo River system	(Glaubrecht and Strong 2007)			
- Pachychilidae	+	-	-	?
Pseudopotamis on Torres Strait Islands	(Glaubrecht and Rintelen 2003)			
Tylomelania on Sulawesi	+	-(+[4])	-/+[4]	?
	(Rintelen *et al.* 2004, 2007)			
Brotia, Kaek River in Thailand	+	+	+/?[5]	?
	(Glaubrecht and Köhler 2004)			
Madagasikara on Madagascar	+	-	-	?
	(Köhler and Glaubrecht 2010)			

[1] Note that Glaubrecht and Strong (2007) and Glaubrecht (2008a) concluded independent colonization events in the paludomids of this lake, suggesting the existence of at least two distinct species flocks within the lake. Nevertheless, both have been found as closely related within the monophyletic African paludomids.

[2] Note that only in case of *Lavigeria* rapid speciation is likely. See Wilson *et al.* (2004) for details on the phylogeny.

[3] Note that within the endemic *Tylomelania* there is evidence for four independent monophyletic clades having colonized the lake systems.

[4] Only verified so far for the case of three syntopic species in the Balocci Valley, SW Sulawesi (Rintelen *et al.* 2007).

[5] Note that Köhler *et al.* (2010) verified common ancestry and rapid speciation, but not phenotype-environment correlation.

(see review in Glaubrecht 1996) versus neutralism without any role of adaptation (Gorthner 1992). Escalation, *i.e.*, predator-prey coevolution, has been positively tested for crabs and snails in Lake Tanganyika (West *et al.* 1991, West and Cohen 1994, 1996) and in the Malili lakes on Sulawesi (Rintelen *et al.* 2004).

For a long time, intralacustrine geographical separation, as a version of (micro-)allopatric speciation, was the generally assumed (though rarely explicitely stated) null hypothesis for the origin of the diversity within a lake. On the other hand, any test of the involvement of natural selection and adaptation, *i.e.*, an ecological component to speciation, was essentially lacking. According to the ecological theory of adaptive radiation (Schluter 1996, 2000a, 2000b, 2001), species flocks are the outcome of divergent natural selection resulting from resource competition (see the first section of this paper for more details). Thus, although the concept of adaptive radiation has enjoyed wide recognition and interest, most alleged cases, and even textbook examples of adaptive radiation were hardly tested with good models.

Only recently has the definition of adaptive radiation been rigorously translated into formal criteria (Schluter 2000a). Accordingly, the two principal components of adaptive radiation are rapid, multiple splitting of a lineage (speciation) and the origin of new ecological and phenotypic forms in each speciation event. For these components Schluter (2000a) proposed four criteria, which should be tested to confirm that the system under consideration indeed represents an adaptive radiation: (1) monophyly (or common ancestry), (2) rapid speciation, (3) phenotype-environment correlation, and (4) trait utility; these provide two phylogenetic and ecological criteria each (see Table 2).

While the latter two criteria are most frequently neglected, and in particular, trait utility is extremely difficult to test even in well-known systems, the first two criteria are more readily testable today, but have not been applied rigorously in many cases yet. As speciation and adaptive radiation is widely considered to be one of the most important processes in evolution, this lack of basic data for most potential candidates is a major obstacle for our understanding of some of the most intriguing evolutionary phenomena. I review and have compiled in Table 2, for freshwater Cerithioidean model systems, the available studies, their results and implications, aiming at explicitly evaluating Schluter's four criteria for speciation and radiation.

Lake Tanganyika: The "thalassoid" species flock in paludomids

The thalassoid, *i.e.*, marine-like, Cerithioidean gastropods in East African's Lake Tanganyika provide one of the iconic examples of a spectacular endemic radiation. Although characteristic of the fauna of this Rift lake, the species flock long remained enigmatic with respect to its origin and evolutionary history of its constituent members, as reviewed in Glaubrecht (2008a). Evaluating Schluter's criteria, I will focus on the monophyly of the entire Tanganyikan species flock and its potential riverine ancestry, as well as the speciation mode and aspects (Table 2).

Common ancestry

All former studies (Michel *et al.* 1992, 2004, Michel 1994, 2000, West and Michel 2000) have assumed (i) that these gastropods are Thiaridae, and (ii) an *in situ* radiation within Lake Tanganyika, explicitly or implicitly hypothesizing that the riverine *Potadomoides* Leloup, 1953 from the Congo River drainage is ancestral to the entire Tanganyikan radiation. In contrast, Glaubrecht (2008a) and Glaubrecht and Strong (2007) have shown based on morphological and molecular phylogenetic evidence (see also Wilson *et al.* 2004) both from the lake radiation itself and a study of the taxa in adjacent river systems, that (i) the thalassoid species in Lake Tanganyika are Paludomidae instead of Thiaridae (Table 1), (ii) that the lake most likely provides an evolutionary reservoir for several ancient confamiliar African lineages, and (iii) that the uterine brooder *Potadomoides* represents the adelphotaxon to only the uterine brooder *Lavigeria* Bourguignat, 1888 (plus the oviparous *Vinundu* Michel, 2004), but not the entire essentially oviparous thalassoid species flock. Based on our phylogenetic analyses, we anticipate that the widely distributed paludomid *Cleopatra* most likely represents the sister taxon to most if not all the remaining thalassoid species in Lake Tanganyika.

Rapid speciation

Accordingly, at least two separate paludomid lineages have once independently colonized Proto-Lake Tanganyika, with only *Lavigeria* exhibiting subsequent radiation and increased speciation events within the lake, whereas most other thalassoid taxa are species-poor or even monotypic (Glaubrecht 2008a). While West and Michel (2000) suggested an "explosive origin" of the entire thalassoid gastropod radiation, we found in our phylogenetic analyses only in the case of *Lavigeria* evidence hinting at rapid speciation (Wilson *et al.* 2004); see Table 2. The viviparous *Lavigeria* has repeatedly been discussed as being especially prone to speciation, assuming that brooders have highly isolated populations more likely to diverge genetically and, thus, to eventually speciate (*e.g.*, Michel *et al.* 1992, Michel 1994). As much as this may or may not be true in general, it is difficult to confirm in the case of the thalassoid gastropods and *Lavigeria* in particular, since essential data on chorology (occurrence, range, and dispersal) and population genetics of the various morphospecies currently differentiated and/or re-erected from former Bourguignat's names by Michel and co-workers are available to only a limited degree (see discussion in Glaubrecht 2008a).

Ecology

Unfortunately, as the taxonomy and systematics of the latter genus has not yet been formalized, we lack sufficient morphological and/or ecological data to allow sound judgement on the question of trophic specialization or other differential adaptation under natural selection of individual taxa of this species flock. In several *Lavigeria* species, conchological congruence with substratum type and specific habitat preferences seems to occur, as reviewed in Glaubrecht (2008a). For example, it was hypothesized that rock-dwellers might be more subject to barriers to dispersal und eventually speciate (Michel *et al.* 1992, Michel 1994). However, to date we can only speculate that specific adaptations in *Lavigeria* could have played a role in intralacustrine speciation and adaptive radiation. In contrast to our studies on *Tylomelania* on Sulawesi (see below), we lack sufficient reliable data on specific radula morphologies and trophic specialization in *Lavigeria* being correlated with specific habitat and/or even food preferences.

Therefore, we find again in the case of Tanganyikan paludomids that the molecular genetic approaches have helped to resolve at least the basic patterns of genealogical relationships and ancestry (phylogenetic criteria, see Table 2). However, we still lack insight into the details of the species flock's geography and in particular ecology, as an explicit study on patterns and mode of speciation has not been undertaken yet. To date it remains equally likely that speciation in Lake Tanganyikan paludomids is promoted either by intralacustrine geography along the inshore environments inhabitated by *Lavigera* and other thalassoid snails, or by adaptation to ecological factors and other selective forces.

Central lakes, Sulawesi: *Tylomelania*'s "evolutionary ecology play"

In the central mountain region of the Indonesian island Sulawesi there are two major ancient lake systems, the Malili lakes (Danau Matano, Mahalona, Towuti, Lontoa, and Masapi) and to the northwest, isolated from the former, solitary Lake Poso. These lakes harbour a diverse array of endemic species flocks, comprising Cerithioidea gastropods and bivalves (Corbiculidae), atyid shrimps (*Caridina* H. Milne Edwards, 1837), crabs (Parathelphusidae, Sundatelphusidae) and fishes (Telmatherinidae in the Malili lakes, Atherinidae in Lake Poso) (see Glaubrecht 2010a). In contrast to lakes in the Malili system, the invertebrate fauna of Lake Poso is more disparate and diverse, containing an endemic, cementing corbiculid (erroneously named a distinct genus *Pososirea* Bogan and Bouchet, 1998, see details in Rintelen and Glaubrecht 2006) and an endemic, unusual lymnaeid snail (*Miratesta* Sarasin and Sarasin, 1898, see Albrecht and Glaubrecht 2006, Albrecht *et al.* 2010).

While it was long assumed that this gastropod species flock arose by adaptive radiation (Wesenberg-Lund 1939, Brooks 1950, Davis 1982), the exact nature of this mechanism remained elusive due to lack of data. The few existing species descriptions were typological, sampling density rather low, and data on biology lacking altogether. Following century long neglect, the endemic evolution of the pachychilid gastropods *Tylomelania* Sarasin and Sarasin, 1897 on Sulawesi now offers a most instructive model case for speciation mechanisms and truly adaptive radiations (Table 2), as documented and discussed at length in Rintelen *et al.* (2004, 2007, 2010), Rintelen and Glaubrecht (2005) and Glaubrecht and Rintelen (2008a). A systematic revision of species, including morphology and a molecular phylogeny of *Tylomelania,* provided the prerequisite of a more thorough speciation study testing allopatry, ecology (*e.g.*, habitat preference) and specialization (radular morphology). In addition, the systematic position and biology of the Sulawesi pachychilids have been clarified in relation to other confamiliar Southeast Asian taxa (*e.g.*, Glaubrecht and Rintelen 2003, Köhler *et al.* 2004, Rintelen and Glaubrecht 2005, Köhler and Dames 2009, Köhler and Glaubrecht 2010).

Common ancestry

All 76 pachychilids (44 species plus *ca.* 32 yet undescribed) endemic to Sulawesi have been shown to belong to the viviparous *Tylomelania,* with currently 23 fluviatile (9 plus *ca.* 14 undescribed) and 53 lacustrine species, of which 25 species are in the Lake Poso drainage (with only 7 formally named to date) and 28 species are formally described from the Malili lakes. Monophyly of this clade has been proven in several phylogenetic analyses cited above. Glaubrecht and Rintelen (2003) have shown that the only other pachychilid, *Pseudopotamis* Martens, 1894, disjunctly occurring in the Austral region with two apparently relictual species on the Torres Strait Islands between New Guinea and Australia, is the adelphotaxon of the *Tylomelania* radiation on Sulawesi. Our historical, biogeographic scenario discussed in the former paper suggested an at least Miocene age and vicariant event at the origin of these two lineages. From a molecular clock approach in Köhler and Glaubrecht (2010), it can be concluded that the age of the *Pseudopotamis* and *Tylomelania* lineage is about 30 to 35 my, with the two taxa having separated at about 15 to 20 my ago. However, the actual radiation in rivers and lakes on Sulawesi might be (much) younger (Table 2).

Rapid speciation

Again, the molecular data provide a successful test of both phylogenetic criteria *sensu* Schluter (2000a). Evidence from mitochondrial DNA analyses (COI and 16S, with a total of

1535 bp) with strong support for four clades, suggests four independent colonization events in the Sulawesi lakes, three of these in the Malili lakes plus one in Lake Poso, with riverine taxa being identified as sister groups to three lacustrine clades (Rintelen *et al.* 2004, 2010). The radiations differ considerably in the extent of their diversification, with species numbers and morphotypes, respectively, ranging from three to 25 (Malili 1 clade: 13 spp.; Malili 2: 12 spp.; Malili 3: 3 spp.; and Poso: 25 spp.). Rapid cladogenesis can be inferred from short branch lengths between many basal nodes within each of the four clades (Fig. 3). A separate invasion of Poso and Malili is to be expected, given that these lake systems were never connected. However, it is surprising that colonization took place independently in different ancestral lineages in the three major lakes of the Malili system, which are directly connected by rivers. The high level of support for the four major lake clades contrasts with virtually no resolution at the species level for *Tylomelania*. All lacustrine morphospecies for which more than one specimen or population has been sequenced appear polyphyletic in the molecular phylogeny (Rintelen *et al.* 2004, 2007, Glaubrecht and Rintelen 2008a). This lack of resolution is even more remarkable since there is no lack of genetic structure *per se* in the data, *i.e.*, there are several well supported subclades within three of the four major lake clades. While this pattern may be caused by several factors (see discussion in the general section), a pivotal role for introgressive hybridization is indicated by genotype-phenotype mismatches and preliminary nuclear amplified fragment length polymorphism (AFLP) data (Glaubrecht and Rintelen 2008a, Rintelen *et al.* 2010).

Ecology

Nevertheless, as each colonization event was followed by diversification into an array of morphologically distinct and ecologically specialized species, they provide evidence for the fulfilment of the two remaining ecological criteria for an adaptive radiation *sensu* Schluter (2000a), see Table 2. We found that radular morphology is highly diverse in all lacustrine lineages of *Tylomelania*, with three to six phenotypes found in each clade (Rintelen *et al.* 2004, 2010, Glaubrecht and Rintelen 2008a). In contrast, radular morphology is highly conservative in riverine species. As radular differences having been demonstrated to be indicative of food and substrate preferences (*e.g.*, Hawkins *et al.* 1989), trophic morphology and substrate were found to be highly correlated in all clades. We propose that this allowed several species to coexist sympatrically and syntopically.

All species in the major lakes of the Malili system and Lake Poso are specialized on either soft substrates (mud, sand) or hard substrates (rock, sunken wood), with about 50% of species occurring on either substrate category (Rintelen *et al.* 2007). Some species are specialized on an even finer scale, *e.g.*, restricted to wood only. Hard substrate taxa in particular show a wide range of mostly substrate- and species-specific radular forms, and there is generally a tight correlation between enlargement of radula denticles and hard substrate. For detailed description and examples see Glaubrecht and Rintelen (2008a) and Rintelen *et al.* (2010), based on data presented in Rintelen *et al.* (2004, 2007). The parallel, substrate-specific occurrence of most radula forms in both ancient lake systems on Sulawesi supports a tight link between radular morphology and substrate. These observations suggest a functional role for differences in trophic morphology, although a detailed understanding of the underlying mechanisms requires further investigation. In concert with the radular differentiation, habitat specialization (substrate and to a lesser degree depth preferences) enables that more than five, possibly up to seven, species of *Tylomelania* coexist at localities with sufficiently structured habitats. This finding becomes even more striking when contrasted with the situation in rivers in general (see below) and with riverine *Tylomelania* on Sulawesi in particular, where only one single species is regularly found, with the exception of sympatric occurrence of three species in the Balocci Valley in Southwest Sulawesi (Table 2). Again, our data suggest a strong role for ecological factors in the diversification and possibly even speciation of *Tylomelania*. We anticipate that parallel situations in several of the lake species provide ideal models to further test for ecological speciation.

However, two factors need to be taken into consideration. First, our detailed studies on selected species and species complexes reveal a more complex and complicated situation. Second, it should not be overlooked that in many cases the species distribution in lacustrine *Tylomelania* is clearly allopatric, *i.e.*, species are essentially confined to one of the three major lakes within the Malili system, where they occur syntopically with other clearly distinct species. This pattern of allopatric occurrence—and presumably speciation—is strong evidence for involvement of geography in speciation within lakes. In addition, we found indications in adult shell and radula characters, and molecular genetics for the possible action of introgressive hybridization in *Tylomelania sarasinorum* Kruimel, 1913 and *T. bakara* Rintelen and Glaubrecht, 2003 in Lake Towuti, as well as many (if not all) species in Lake Mahalona, between the Matano and Towuti lakes (Glaubrecht and Rintelen 2008a). More strikingly, specimens of one fluviatile species are found terminally in one lacustrine clade basically restricted to Lake Towuti, thus similar to the telmatherinid fishes (see Glaubrecht 2010). However, whether hybridization is involved during the speciation process in *Tylomelania* in general has to await more comprehensive data from our ongoing AFLP study (Rintelen and Glaubrecht, unpubl. data).

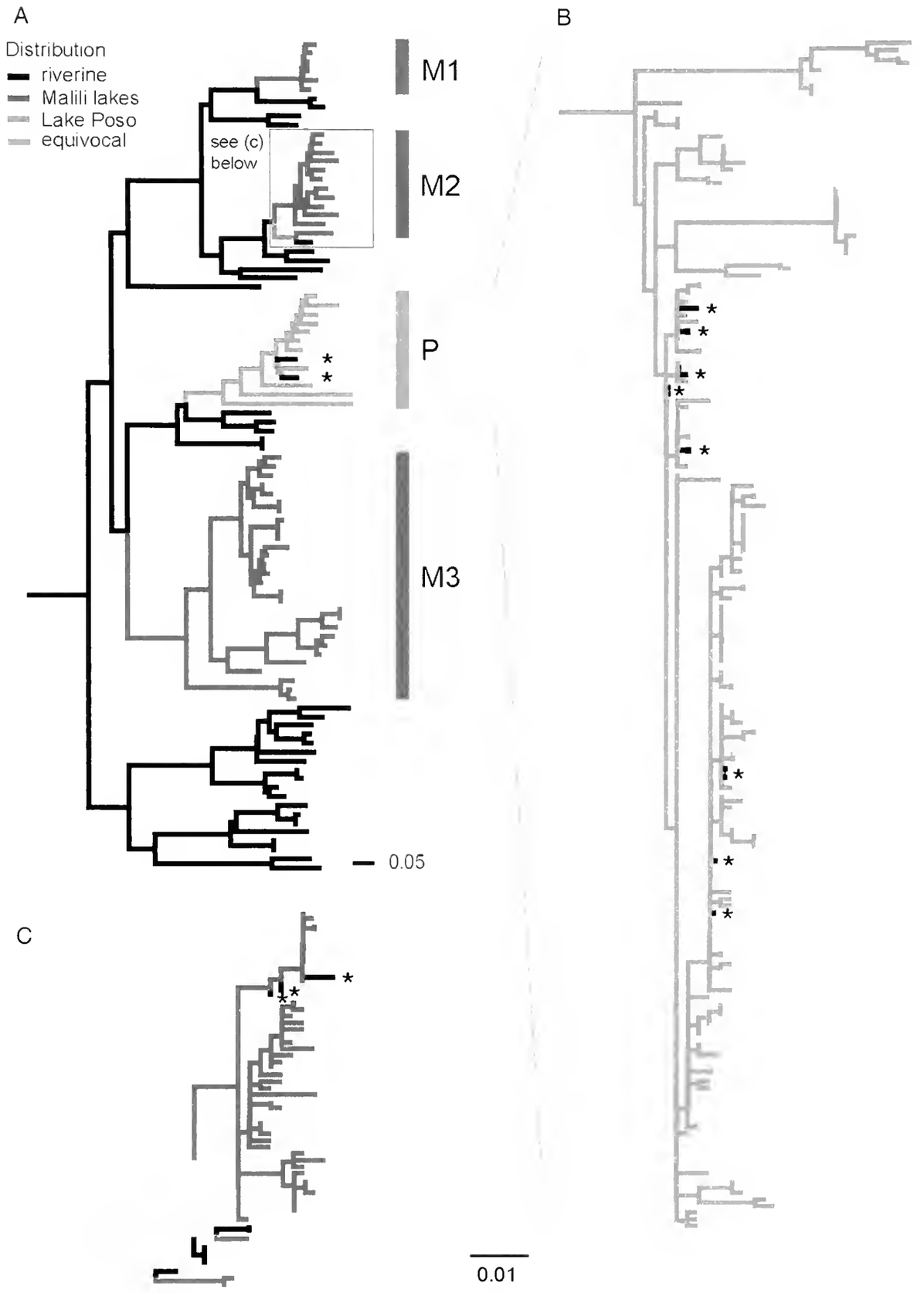

Figure 3. Molecular phylogeny of the pachychilid gastropod *Tylomelania*, endemic to the Indonesian island of Sulawesi, based on Bayesian inference analysis using mtDNA sequences (COI and 16S, with a total of 1535 bp). A, Phylogram with Malili (M) and P (Poso) lakes taxa, marking four lacustrine clades and independent colonization events. The others are riverine species, with asteriks indicating riverine taxa in terminal position within lacustrine clades. Modified from Rintelen *et al.* (2004). B, C, Detailed sections of BI phylogram based on 660 bp of the COI phylogram for the Poso clade (B) and the Malili 2 clade (C). Reproduced from Rintelen *et al.* (2010).

Contrasting lacustrine species flocks: riverine radiations in Cerithioidean gastropods

Riverine radiations have not attracted the same attention as speciation in lacustrine settings. Rivers around the world hold species-rich assemblages of molluscs, for example, among Cerithiodea, the endemic Pleuroceridae in Tennessee and the Coosa-Cahaba Rivers in the southeastern USA, or the Pachychilidae in Central America (Table 1). Unfortunately, details of their phylogeny, phylogeography and evolution are largely unknown. The few cases among gastropods have been recently reviewed in Glaubrecht and Köhler (2006), and the reader is refered to the literature therein. Only rarely is there convincing evidence for adaptive radiation to have taken place *in situ* in lotic habitats. Nevertheless, riverine cases can shed additional light on the intrinsic and extrinsic properties involved in radiations and the origin of diversification in general, helping to elucidate the evolution of intra-lacustrine species flocks.

As is evident from comparison, for example, both the Congo River drainage and Lake Tanganyika provide stable evolutionary systems, both in their own right and with distinct inherent environmental dimensions. Emphasis has shifted recently to non-lacustrine settings, with studies focussing on various Cerithioidean gastropods in rivers, as reviewed below (see also Table 2). Intralacustrine radiations should not to be seen as evolutionary dead ends, rather, it is possible the repeated switches of limnic taxa between riverine and lacustrine habitats during their evolution guaranteed their survival, and provided ample opportunities for speciation and radiation under various conditions.

Congo River system: *Potadomoides* as Tanganyican "ancestor"?

The riverine genus *Potadomoides* from the Congo River drainage has proven to be crucial for our understanding of the lacustrine Tanganyikan radiation. Following Brooks (1950: 150) speculation that the thalassoid snails are presumably derived from fluviatile ancestors present at the lake's genesis, Brown (1994: 530) proposed that *Potadomoides* has the strongest claim for consideration in relation to this ancestry (see above).

Common ancestry

Cladistic analyses of morphological data verified the long proposed sister group relationship of lacustrine species of *Lavigeria* (plus *Vinundu)* endemic to Lake Tanganyika and the fluviatile *Potadomoides* from the Congo drainage system, subsumed now under Nassopsinae Kesteven, 1903 (= Lavigeriinae Thiele, 1925) (Glaubrecht 1996, 2008a, Glaubrecht and Strong 2007). This fluvio-lacustrine clade, uniting two lacustrine taxa and the fluviatile *Potadomoides*, apparently represents an early independent lineage of East African paludomids that until recent times survived in and adjacent to Lake Tanganyika. Consequently, while *Potadomoides* certainly does not serve as a suitable model for the majority of the thalassoid gastropods, it might serve as a model for an African paludomid with the potential to colonize not only riverine but also lacustrine habitats.

Rapid speciation and ecology

The species composition was long unresolved, with six species originally described to *Cleopatra* and later transferred to Leloup's new genus (Glaubrecht and Strong 2007). For the recent re-transfer of *C. broecki*, see Glaubrecht (2010c). In contrast to the type species *P. pelseeneri* Leloup, 1953 which is endemic to the Malagarasi delta, the three other congeneric species are restricted to the upper reaches of the Congo River in eastern Zaire. While it is apparent from the shell morphology of these remaining four taxa as well as their anatomy (in particular reproductive systems) that *Potadomoides* might possess the potential for a profuse radiation in Lake Tanganyika, we lack data as to the age and mode of speciation. Since *Potadomoides* is sister to *Lavigeria,* the molecular analysis of thalassoid paludomids hints at a more recent event. The largely conservative radula morphology clearly supports *Potadomoides* as the adelphotaxon of *Lavigeria* plus *Vinundu.* However, due to the lack of ecological observations from their riverine habitats, we are unable to make inferences as to the mode of speciation, or Schluter's (2000a) criteria. Unfortunately, we lack recent collections of this riverine gastropod, and suitable material for molecular genetic studies. No large-scale exploration of limnic habitats in the Congo Basin has been undertaken for several decades. However, equal to great lakes such as Tanganyika and Malawi, the Congo River and its easternmost tributaries have a species assemblage (not only of unique molluscs) that deserves more attention in the future.

Kaek River, Thailand: unraveling a riverine radiation of *Brotia*

Davis (1982) was first to observe that when *Brotia* is found in rivers in SE Asia, there is usually one species, or two at the most. The only exception to this rule is a small radiation in the Kaek River drainage in north central Thailand, part of the Nan-Chao Phraya system. An exceptional assemblage of morphologically distinct, viviparous species of *Brotia* is endemic to this river system, with seven recognized species exhibiting a remarkable degree of conchological disparity, and with syntopic occurrence of three to four species that are ecologically separated by habitat preferences and trophic specializations (Glaubrecht and Köhler 2006, Köhler *et al.* 2010).

Common ancestry

Based on morphological, molecular phylogenetic data, and phylogeography using mtDNA gene fragments (COI and

16S), it is evident (i) that all species from the Kaek River and adjacent drainages form a monophyletic group of *Brotia* within a large Thai clade, and (ii) that the origin of this Kaek River clade derives from a Mekong River ancestor. In light of the palaeo-hydrological situation of Pleistocene stream capture in accordance with alignments of post-Himalayan river systems, we proposed that together with other faunas pachychilid snails could have been captured and added to the fauna of rivers from the Mekong via the Loei to the Kaek and thus Chao Praya. This would explain the origin of the Kaek River flock in a taxon from the Mekong drainage. Likewise, it is possible that during the complex geological history, with its wide spectrum of tectonic changes affecting the major drainage systems, rivers or at least some of their sections, could have stranded as new lakes, isolating faunas for a million of years or more, and offering amble opportunity for lacustrine radiations. For a more indepth discussion of this and the evolutionary aspects see Glaubrecht and Köhler (2006), and Köhler *et al.* (2010).

Rapid speciation

The relatively shallow topology of the Kaek River gastropods, and the lack of significant resolution in our molecular phylogenies, *i.e.*, the mismatch of gene and species trees, imply a relatively recent origin of this intra-riverine radiation and, consequently, rapid speciation with a high degree of morphological divergence. Thus, again rapid cladogenesis is inferred from short branch lengths within the clades. Several other cases are known where morphologically distinct (sympatric) species have evolved without marked changes in the mitochondrial genome. There are alternative explanations employed in view of lacking greater resolution and flat topology such as incomplete lineage sorting, hybridization which can only be falsified by additional molecular genetic data (see above). Nevertheless, we interpret these data as fulfilment of Schulter's two phylogenetic criteria in the case of the seven *Brotia* species from the Kaek River (Table 2).

Ecology

More difficult to verify is whether there is any phenotype-environment correlation in these unusual *Brotia* species. Along the Kaek River, the syntopic occurrence of up to three or more species was documented during field work. Available data from our first study (Glaubrecht and Köhler 2006) hinted at a similar phenotype-environment correlation as in lacustrine *Tylomelania*. In several locations along the Kaek River, preliminary work indicated that habitat preferences of individual *Brotia* species correlate with their radula morphology. Again, trait utility could not be tested (Table 2). However, Köhler *et al.* (2010) are more cautious as to the ecological adaptation criterion, suggesting that there is no such correlation and/or trophic specialization. More sophisticated studies are necessary to establish the eminent role of spatial factors in evolution, highlighting the importance of local, adaptive divergence for geographical patterns of speciation. Representing a riverine radiation of a monophyletic species flock with presumably relatively recent diversification, two questions still remain unresolved: (i) the origin of the Kaek River species of *Brotia* from a colonist derived from the Mekong drainage and subsequent river capture providing a possible hydrological explanation, and (ii) the causation for an *in situ* speciation along the river and the contribution of geographical (*i.e.*, historical) versus ecological factors.

Madagascar: rediscovering a radiation in *Madagasikara*

Recently, an overlooked riverine radiation of Cerithioideans has been uncovered on Madagascar. Renowned for its rich and diverse biota that evolved during lengthy isolation, the continental island also harbours pachychilid taxa that were traditionally attributed to *Melanatria* Bowdich, 1822. This name, however, is an objective synonym, thus not available and replaced now by the new genus name *Madagasikara* Köhler and Glaubrecht, 2010. A considerable number of formal species-group names have historically been affiliated with this group, suggesting a considerable degree of morphological diversity mainly in the shell, as this was the feature traditionally emphasized by conchologists. While recent authors stated that this diversity only mirrors infraspecific variability of one or two valid species, other studies of pachychilid gastropods revealed that overemphasis on intraspecific variability in freshwater snails might result in a significant underestimation of species diversity. Taxonomic revisions using modern evolutionary systematic approaches are needed to assess species limits and provide the framework for the study of speciation and radiation.

Common ancestry

By analyzing morphology and molecular genetics (based on a mtDNA fragment of the 16S gene), we found that the diversity of this group has been underestimated, resulting in the description of three new species in addition to the two species previously recognised. Using a mitochondrial phylogeny that includes all currently accepted pachychilid genera and a strict molecular clock approach with two alternative calibrations, we found that the origin of the family Pachychilidae was no more than 50 mya, whereas the origin of the Madagascar lineage is estimated as between 15-31 mya, approximately concurrent with the colonization events in a number of other Madagascar animal taxa.

Rapid speciation

With the differentiation of the five named Madagascan species starting probably not before 10 mya, the pachychilid

radiation on the island appears to be not older than 3 to 5 mya. The topology of this section of the molecular phylogeny hints at a rapid radiation event that led to the colonization of all major river systems of the island (with the exception of the drier southwest). As the *Madagasikara* species of our phylogeny have non-overlapping occurrences, in contrast to the exceptional riverine radiation reported for Kaek River pachychilids, we conclude allopatric speciation in their case. However, given the paucity of material and imprecise localities for older museum collections, an explanation of the evolutionary history of *Madagasikara* and regional patterns of speciation is premature. The diversity and fragmentation of freshwater habitats on this island is associated with considerable levels of local endemism, and in many freshwater groups the majority of species are restricted to single rivers or creeks.

Ecology

We found five species of *Madagasikara* to inhabit Madagascar, with just one species occuring in any one river or river system. While the east coast with its short rivers is inhabited by two species, the remaining species occur in restricted regions along the north and northwest of the island. Thus, not only is there an unrecognised radiation in *Madagasikara* with species-level differentiation in particular along the drainages of the northwest of the island, but our analyses also suggest a spatial bi-partitioning along the mountainous N-S axis of Madagascar. A comparison of shell features reveals that species with shells dominated by spiral elements (and axial elements completely lacking) are restricted to the west and north of the island. In contrast, along the east coast, the two species exhibit pronounced axial elements in combination with a strongly stepped shape of the shell. Hardly any other information is available from the field to put this conchological differentiation into perspective. In addition, conservative radula morphology does not hint at trophic specialization as documented in particular in lacustrine *Tylomelania*. Instead it resembles the pattern found for riverine pachychilids on Sulawesi and in Thailand (with the exception of the case of *Brotia* in Kaek River, see above).

Summary: Speciation from a snails' perspective

From the above review it is evident that not only the two different model lake systems and their respective faunal elements, but also riverine settings are distinct with respect to the influence of intrinsic factors (*e.g.*, crucial biological features such as viviparity, dispersal and trophic specialization) versus extrinsic factors (*e.g.*, palaeohydrology, habitat fragmentation, drainage systems). Although lotic systems are generally considered harsh environments (as determined by physical factors and stochastic events reducing densities), they are more permanent on both ecological and evolutionary timescales than most lake habitats. The only notable exception are ancient lakes, which provide ample opportunity for many different organisms to speciate, in contrast to rivers where environmental changes are gradual and do not prevent gene flow. Interestingly, rarely have true riverine radiations been recognized, and the only known cases from Cerithioidean gastropods were discussed above.

As is evident from Table 2, in all cases we have evidence not only for common ancestry but also for a fairly recent origin and rapid speciation with rapid morphological divergence, parallel to the recent diversification of pachychilid species flocks in the ancient lakes on Sulawesi and the *Lavigeria* radiation in Lake Tanganyika. Within each of these clades, a burst of very recent radiations resulted in a partial mismatch of species and gene trees. Apparently, rates of molecular and morphological evolution have been highly divergent in lake species (as opposed to widespread riverine taxa). While there is evidence for trophic specialization linked with differential habitat use within the lakes on Sulawesi, indicating ecological speciation in *Tylomelania*, allopatric mechanisms might also have been involved.

In contrast, the Kaek River with its sympatric and partly syntopic species flock reveals conditions where speciation through allopatric isolation seems implausible, quite to the contrary of *Madagasikara* on Madagascar. This strongly hints at spatial segregation, for example, by trophic substrate specialization correlated with adaptation in radula morphology to different microphagous grazing, as the ultimate trigger of speciation in Kaek River species which, however, remains to be shown. On a finer scale, and exemplified by adequate case studies, future comparative work on the Sulawesi lakes with their relatively large species flocks contrasted with the less species-rich Kaek River group in Thailand and species on Madagascar might help in elucidating these aspects of radiation and origin of diversification.

Although large species inventories and high levels of endemism have been reported for other freshwater gastropods from lotic systems, among them, for example, the North American Pleuroceridae, speciation mechanisms (including allopatric versus ecological speciation models) have not been considered, investigated, or often even discussed. Attempts to evaluate these factors suffered from lack of insight into systematics and morphology (*i.e.*, the reproductive biology) of the limnic gastropods involved, and how crucial differences in the geological setting were, such as *e.g.*, rifting in the *ca.*12 myr old Lake Tanganyika versus Sulawesi's composite terrane origin with *ca.* 1-2 myr old lakes.

In summary, we arrive at the following conclusions: (i) Allopatry: The allopatric speciation model offers a well-supported starting point for the explanation of the existence of species flocks in *Tylomelania* at least in the Malili lakes (but not Lake Poso) on Sulawesi. The same is most likely for

riverine cases, such as *Potadomoides* in the Congo River and *Madagasikara* on Madagascar. (ii) Ecology: Substrate preference found in most lacustrine (and riverine) species as well as trophic specialization studied so far in more detail in some *Tylomelania* species hint at the importance and relevance of ecological factors responsible for the coexistence of species. However, we lack clear evidence that competition-driven specialization was the ultimate cause of speciation, or that adaptation and trophic morphology were drivers for the radiation in the case of the *Brotia* species from the Kaek River. (iii) Complexity: Exemplary studies of individual species, in particular those of lacustrine *Tylomelania* and riverine *Brotia* from Kaek River revealed that contincency plays a role and that the evolutionary history of freshwater gastropods in lacustrine as well as riverine settings is more complex than assumed earlier. As we have so far utilized essentially the more easily available mitochondrial genes, other potentially relevant factors and processes that drive speciation and radiation (*e.g.*, ecology, adaptation, natural selection, hybridization) can often only be assumed, and these need to be tested in more detail in the field.

CONCLUSION

"A scientific revolution that makes no difference to everyday scientific work seems an odd sort of revolution." Endersby (2009: 1499)

We came a long way to understand how new species originate, which remained Darwin's "*mystery of mysteries*". Since speciation is the underlying mechanism for radiation, it is the ultimate causation for the biological diversity of life that surrounds us. While the great importance of speciation and of adaptive (and non-adaptive) radiations for biodiversity is widely accepted, our understanding of the processes and mechanisms involved is still limited. Any broader generalization needs to be based on the accumulation of more evidence from additional case studies, which freshwater gastropods can offer in many different environmental settings. In addition, today there is a variety of current approaches in evolutionary research to study speciation.

The use of modern techniques from molecular biology, bioinformatics and systematic phylogeny allow reconstructing the relationships of organisms, the course of evolution and its underlying causation. Many model cases using these techniques show the progress and dynamics of this kind of research. At the same time it has become clear (i) that the phylogenetic patterns revealed by phylogenetic trees (irrespective of the individual genes and algorithms used) offer only a first approach and proxy for species richness, speciation mode and (adaptive) radiation, (ii) that palaeontological patterns often lack the temporal-spatial resolution to resolve speciation and detect the respondible mechanism, (iii) that models such as Cerithioidean gastropods in insular, but rarely in other "natural laboratories" provide us with species flocks that allow for detailed and sophisticated studies of these mechanisms.

Finally, I conclude that allopatric speciation should still be used as a null-hypothesis for testing and falsifying non-allopatry by studying ecological and other relevant factors. It remains to be shown whether and to which degree ecological (and sympatric) speciation is involved in the origin of species flocks and whether for example trophic specializations can provide evidence for natural selection playing an ultimate role in the process of speciation and radiation. Although these phenomena may no longer be the same "*mystery of mysteries*" for us as it once was for Darwin, our answers are not completely satisfactory yet, with many questions still remaining unanswered.

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Speciation in modern marine bivalves (Mollusca: Bivalvia): Insights from the published record*

Paula M. Mikkelsen

Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, New York 14850, U.S.A.

Correspondence, P. M. Mikkelsen: pmm37@cornell.edu

Abstract: What can living marine bivalves tell us about speciation in the marine environment? Three sets of literature data on Recent marine bivalves are analyzed for insight into the mechanisms behind bivalve speciation processes. (1) A dataset of all marine bivalves described as new to science during the years 2000-2009 (381 species in 135 published papers) reveals that malacologists are still describing undiscovered biodiversity, based largely upon newly collected expedition material. New species include those of both large and small body size (0.86-500 mm, mean 28 mm), from 51 bivalve families, all oceanic basins, and a wide range of water depths (intertidal to 7,333 m, mean 444 m). External shell characters dominate the diagnoses but are increasingly supplemented by anatomical, molecular, and phylogenetic evidence. Endemism is low (2.6%) when stated as such although another 57% of species were described as (thus far) restricted to a particular geographic region, habitat, or both. High percentages of deep-water and otherwise (geographically or ecologically) restricted species, plus several case studies, suggest that physiological specialization, in the form of bathymetric limits, unique dietary adaptations, or host/symbiont associations, plays an important role in setting up barriers to gene flow in marine bivalves. (2) Bivalve species complexes [*i.e.*, closely related, cryptic (possibly sibling) species with obscure morphological boundaries, or highly variable single species] also imply factors involved in ongoing speciation. Seven recently studied marine taxa are presented in which species complexes are either revealed or resolved by molecular data. Apparent barriers to gene flow are in most cases physiological (sympatric), or (in two cases) physical (allopatric), and in one case can be readily broken down by anthropogenic transport. (3) Two recent published phylogenetic analyses are discussed that show (a) disparately sized sister taxa with their synapomorphies (glochidia in Unionoidea; chemosymbiotic bacteria and mucus-tube feeding in Lucinoidea; cruciform muscle and long siphons in Tellinoidea; aortic bulb in Veneroidea) as recognized innovations that facilitated radiation of the more species-rich sister, and (b) polytomies in Lucinoidea that suggest rapid ongoing evolutionary change in several clades. Together these three sets of published data defeat the concept of a Marine Speciation Paradox in bivalves—speciation clues are merely subtler in marine bivalves and most often act at the physiological, rather than physical, level.

Key words: taxonomy, species complex, phylogeny, allopatry, sympatry

The marine environment challenges the study of speciation because, despite high diversity in habitat, body form, lifestyle, and taxonomic category, (1) the marine realm has less effective barriers to dispersal (at least in the more traditional, terrestrial sense; one of the few exceptions might be geminate species associated with rise of the Isthmus of Panama, see Smith *et al.* 2006, Marko and Moran 2009 for recent studies) and therefore fewer opportunities for allopatric isolation (Vermeij and Grosberg 2010), and (2) the planktonic larvae produced by most marine organisms have the potential for wide dispersal and thus to maintain high levels of gene flow between populations. This is called the Marine Speciation Paradox (Bierne *et al.* 2003). Mollusca, the most species-rich phylum in the sea, provides excellent examples of this pattern. Marine bivalves, the second most species-rich class of molluscs, are particularly vexing. Whereas species of other groups of molluscs—especially landsnails and freshwater bivalves—are often restricted to one hillside or island or lake, marine bivalves are often widely distributed in what is at least superficially a more-or-less continuous, homogenous environment, and the mechanisms underlying their radiation are more difficult to comprehend. Even marine gastropods are more straightforward—unlike bivalves, many gastropods have reproductive or feeding specializations that lend themselves readily as isolating mechanisms (see Allmon and Smith 2011, Krug 2011).

To look for insight into marine bivalve speciation, especially toward resolving the presumed paradox, I have examined new species descriptions (plus the supporting data behind them), recently studied species complexes, and phylogenies published during the past decade to examine what these data can reveal (if anything) about ongoing speciation in marine bivalves. The following questions are foremost: What kinds of marine bivalves are we currently

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describing as new? Are there any groups conspicuously missing from this dataset? What criteria (morphological [shell, soft anatomical], molecular, phylogenetic) have been used to define these species as new? Do these new species descriptions reflect recently isolated species, or something else? Can we identify any groups of marine bivalves that are currently undergoing rapid speciation? Can we identify mechanisms for marine bivalve speciation in the current era?

MATERIALS AND METHODS

New species descriptions of Recent marine bivalves for the years 2000-2009, as a sample time interval in the current era, were compiled from *Zoological Record* via the online Thomson Reuters database "Web of Knowledge" (see http://wokinfo.com/), accessed through Cornell University Library. Each species was tabulated in a simple spreadsheet according to date of publication, family, geographical, and depth distribution (the latter not differentiating between live- and dead-collected specimens), maximum recorded size, endemicity (or "restriction" in geographic distribution or habitat), distinguishing characters from nearest relatives, the source of the new material (*e.g.*, new collections/expedition, taxonomic revision, etc.), and whether the original description included anatomical data, molecular data, and/or a supporting (phylogenetic or another type of numerical) analysis. Shell characters distinguishing new species from nearest relatives were further broken down into 12 categories: size, shape, color, sculpture, thickness, inflation, muscle scars/pallial line, lunule/escutcheon, hinge, ligament, prodissoconch/umbones, and periostracum. The data presented herein were generated by simple resorting of the spreadsheet according to particular columns.

RESULTS AND DISCUSSION

New species descriptions 2000-2009

New species of molluscs are being described every month, if not every week. The rate of new species descriptions per year in malacology has indeed been high since 1758 and relatively constant for at least the past century (Rosenberg 2009). For this part of the analysis, new Recent marine bivalve descriptions from the malacological literature were tabulated for the years 2000-2009, totaling 381 species by 118 authors (64 first authors) in 135 published papers (see Appendix). The mean number of species described per year during this time interval was 38.10 ± 21.59 *SD* (standard deviation) [maximum (2002): 82 in 18 papers; minimum (2000): 12 in 7 papers]. The mean number of species described per paper during this time interval was 2.83 ± 3.58 *SD* [maximum (2002): 4.56 ± 5.41; minimum (2000): 1.71 ± 1.25].

Source of the material

Malacologists are describing new species based largely (243 of 381 species, or 64%) on four broad categories: (1) new collections (see below; 132 of 381 species, or 35%), (2) taxonomic revisions (either regional or worldwide, at generic or higher taxonomic levels; 98 of 381 species, or 26%), (3) revisions of regional checklists (10 of 381 species, or 3%), or (4) commercial considerations (*e.g.*, aquaculture, harvested species; 3 species: *Tridacna costata* Roa-Quiaoit, Kochzius, Jantzen, Zibdah, and Richter in Richter *et al.*, 2008, *Angulipecten simoneae* Morrison and Whisson, 2009, and *Crassostrea hongkongensis* Lam and Morton, 2003). Many of the important new collections originated from new expeditions (especially the MUSORSTOM Project at New Caledonia and elsewhere, led by Philippe Bouchet at the Museum National d'Histoire Naturelle in Paris, *e.g.*, Dijkstra 2001, 2002, Krylova 2001, Lamprell and Healy 2001, 2002, Glover and Taylor 2007, Lutaenko and Maestrati 2007, ter Poorten 2009) or explorations of extraordinary deep-sea environments such as seamounts, seeps, and hydrothermal vents (*e.g.*, Dijkstra and Gofas 2004, Holmes *et al.* 2005, Oliver and Holmes 2006b).

Nature of the evidence

Malacologists are describing new species from material including live-collected or living animals (179 of 381, or 46%) or (slightly more so) from empty shells alone (202 of 381, or 53%). Although anatomical features when available form part of the original description in most cases (120 of 179, or 67%), these enter into diagnoses against morphologically similar species in only slightly over half of such cases (66 of 120, or 55%). This is not surprising given that bivalve shells are generally recognized as good sources of systematic data sufficient to distinguish them from congeners and other closely related forms (evidenced by the new species descriptions referenced herein). In some cases (*e.g.*, Limidae; Mikkelsen and Bieler 2003), gross soft-anatomical characters at the generic level are remarkably uniform, with diagnoses heavily reliant on external shell features and color of the living animals (both potentially affecting the impact of visual predators). This value of shell characters is also fortuitous for descriptions of fossil bivalves; in both fossils and Recent species known from empty shells alone, muscle scars and pallial lines on inner shell surfaces provide some anatomical information even in the absence of a soft body.

Within the shell characters used in diagnoses ($N = 367$), the description of *Neolepton holmergi* Zelaya and Ituarte, 2003 cited the most categories (6: size, shape, sculpture, inflation, hinge, prodissoconch/umbones, and periostracum). Another 14 species used 5 categories, 47 used 4, 77 used 3, 136 used 2, and 92 used only one shell character (one additional species used one shell character that was unique and not

categorized). The most common single distinguishing categories were sculpture (34 of 72 species, or 47%), shape (17 or 24%), and hinge (10 or 14%). Of the 13 species not citing any distinguishing shell characters, four were distinguished on the basis of habitat, whereas the other nine used anatomical characters. Of the categories, shell shape was most used (217 of 367 species, or 59%), followed by sculpture (205 or 56%), hinge (111 or 30%), size (94 or 26%), prodissoconch/umbones (78 or 21%), muscle scars/pallial lines and shell color (each 47 or 13%), inflation (43 or 12%), lunule/escutcheon (33 or 9%), thickness (32 or 9%), periostracum (27 or 7%), and ligament (18 or 5%).

Although the use of molecular data in molluscan systematics has seen a dramatic increase in the past decade (*e.g.*, Ponder and Lindberg 2008), such criteria are mainly utilized in bivalves at supraspecific levels. Only 9 of the 381 species (2%) were distinguished using molecular evidence, and all of these used molecular evidence in combination with morphological (usually shell) data. Two of the three commercial species were initially distinguished as molecular variants (Lam and Morton 2003, Richter *et al.* 2008), later confirmed by morphology.

Malacologists are generally not describing new species based on phylogenetic analyses. Only 12 of the 381 new species (3%) in seven publications are so supported. Of these, two studies coded morphological data alone (Cosel and Salas 2001 – 5 species; Simone 2009 – 2 species), and five coded molecular data alone (Lam and Morton 2003, Glover *et al.* 2004, Järnegren *et al.* 2007, Richter *et al.* 2008, Rodrigues *et al.* 2008). Although no studies coded both morphological and molecular characters, Cosel (2008a, 2008b) justified several new species of *Bathymodiolus* based on his morphological evidence and a generic-level molecular phylogeny presented earlier (Jones and Vrijenhoek 2006). Another two species were supported as new by a discriminant analysis (Kamanev 2002).

Taxonomic coverage

During the study period, malacologists have described new marine species from 51 of the *ca.* 100 living bivalve families (Table 1). The only taxa conspicuously missing from these descriptions are the Nuculoida and Trigonioida, the former being the more species-rich of the two. These data, however, are clearly highly dependent upon the research interests of active investigators. The greatest representation in this list is the Lucinidae (52 species), which reflects the productive research program of John Taylor and Emily Glover of The Natural History Museum (London) and their associates (Glover *et al.* 2004, Glover and Taylor 2007, Taylor and Glover 2005, Cosel in Taylor and Glover 2005, Cosel 2006). Condylocardiidae, with 33 species, ranks second, based on only three regional revisions by two investigators (Middelfart 2002a, 2002b, Coan 2003). Cardiidae, with 28

Table 1. Taxonomic coverage at the family level by new marine bivalve species described 2000-2009. Number following each taxonomic name is the number of new species described in that taxon during the period under discussion.

Protobranchia			11
Nuculoida		0	
Solemyoida		4	
Solemyidae	3		
Manzanellidae	1		
Nuculanoida		7	
Nuculanidae	1		
Yoldiidae	5		
Neilonellidae	1		
Autolamellibranchia			
Pteriomorphia			89
Arcoida		13	
Arcidae	7		
Glycymerididae	1		
Limopsidae	2		
Philobryidae	3		
Mytiloida		18	
Mytilidae	18		
Pterioida		3	
Ostreidae	1		
Gryphaeidae	2		
Limoida		16	
Limidae	16		
Pectinoida		39	
Pectinidae	15		
Propeamussiidae	14		
Spondylidae	10		
Heteroconchia			
Palaeoheterodonta			
Trigonioida		0	
Heterodonta			281
Carditoida		49	
Crassatellidae	2		
Astartidae	14		
Condylocardiidae	33		
Anomalodesmata		37	
Paralimyidae	1		
Pandoridae	1		
Clavagellidae	2		
Spheniopsidae	6		
Thraciidae	8		
Myochamidae	2		
Verticordiidae	3		
Poromyidae	6		
Cuspidariidae	8		
Veneroida		175	
Lucinidae	52		
Ungulinidae	2		
Thyasiridae	10		
Chamidae	1		
Galeommatidae	22		

Table 1. (Continued)

Veneroida (continued)		
Lasaeidae	1	
Leptonidae	1	
Kelliidae	1	
Montacutidae	2	
Chlamydoconchidae	1	
Kelliellidae	5	
Vesicomyidae	13	
Cyamiidae	3	
Cardiidae	28	
Veneridae	6	
Gaimardiidae	1	
Neoleptonidae	12	
Tellinidae	2	
Psammobiidae	2	
Semelidae	2	
Solenidae	8	
Myoida		20
Corbulidae	4	
Pholadidae	16	
Total	381	

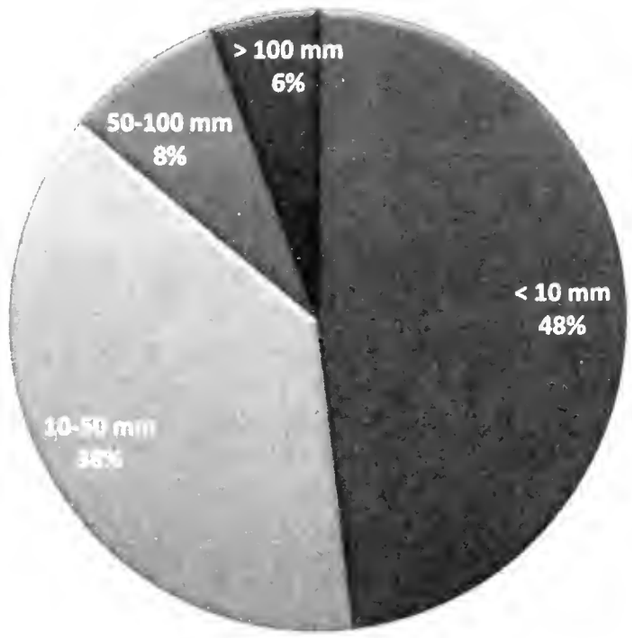

Figure 1. Body size (N = 381, ranging from 0.86 to 500 mm) of new marine bivalve species described during 2000-2009. More than half of the species are >1 cm in maximum dimension.

new species, ranks third from two groups of investigators (Hylleberg and Vidal in Vidal 2000, ter Poorten and Dekker 2002, Vidal 2003, Vidal and Kirkendale 2007). Another major representation is the superfamily Galeommatoidea, with all six families represented by a total of 28 new species from six separate sets of investigators (Simone 2001, 2008, Kamenev 2004b, Middelfart and Craig 2004, Middelfart 2005, Rotvit *et al.* 2007, Lützen and Takahashi 2003, Lützen and Nielsen 2005, Oliver and Holmes 2004).

Body size

Not everything new is small or cryptic. Malacologists are describing bivalves of a wide range of shell size, with the mean shell length (27.99 ± 50.82 mm *SD*) well within "naked eye" visibility, and maximum size for the species ranging from 0.86 mm (*Austrocardiella pouli* Middelfart, 2002; Condylocardiidae) to 500 mm (*Empressostrea kostini* Huber and Lorenz, 2007; Gryphaeidae). Although 185 species (48%) are <10 mm in maximum shell length, the remaining 52% is species of >10 mm, with 54 species (14%) >50 mm in maximum dimension (Fig. 1). So, although small species are certainly prominent among these new descriptions, malacologists are still describing new species of relatively large body size that have heretofore gone unnoticed.

Distribution

Malacologists are describing new species on a worldwide basis, with all oceanic basins represented: eastern Atlantic 60 (28 West Africa, 32 Europe/Mediterranean); western Atlantic 47 (25 North America/Caribbean, 22 South America); eastern Pacific 32 (19 North America/Caribbean, 13 South America); Indo-West Pacific 169 (including 36 New Caledonia); northwestern Pacific 36 (including 26 Japan); Indian Ocean 43; Antarctic 6; and Arctic 8. Many of these reflect the extensive research programs of Philippe Bouchet's colleagues (New Caledonia and other Indo-West Pacific locales), Rudo von Cosel (West Africa), and Luiz Simone and others (Brazil, now recognized as supporting a fauna more distinct from that of the northwestern Atlantic than previously thought). As for taxonomic coverage, this category is highly dependent on researchers and their interests.

Endemism is relative; that is, whether a species is "endemic" or not depends on the context or specific region under consideration. For example, a species can simultaneously be endemic to the Atlantic Ocean, the eastern United States, and Florida, but is not endemic to the Florida Keys if it is also known from Miami. With that qualification, only 2.6% (10 of 381 species) were actually stated as "endemic" in their original descriptions. Nevertheless, another 57% (219 of 381 species) were described as restricted to a particular geographic region (179 of 219 species, or 82%), a particular specialized habitat (12 of 219 species, or 5.5%), or both (28 of 219 species, or 13%). Admittedly, some of these species might not truly be restricted or endemic, but are so far only known from a single place (usually the type locality), such as the Edison Seamount (*e.g.*, *Bathymodiolus anteumbonatus* Cosel, 2008a) or the Argentine Basin (*e.g.*, *Neolepton profundorum* Allen, 2004); such characterizations might be limited by the fact that we

have not sampled in that location often or even more than once. Other such cases are from more widely sampled areas, such as Brazil (*e.g.*, *Solemya notialis* Simone, 2009), where the faunas are relatively well known but perhaps not adequately revised taxonomically. Also included here are species with highly specialized habitat, such as methane seeps (*e.g.*, *Thyasira methanophila* Oliver and Sellanes, 2005), submarine volcanos (*e.g.*, *Gigantidas gladius* Cosel and Marshall, 2003), submarine caves (*e.g.*, *Empressostrea kostini* Huber and Lorenz, 2007), and reducing sediments (*e.g.*, *Lucinoma myriamae* Cosel, 2006), again areas which likely suffer from minimal sampling effort. Still another category here are bivalves that are commensal with other invertebrates, such as sponges (*e.g.*, *Troglodytoconcha carpentariensis* Middelfart, 2005), crabs (*e.g.*, *Arthritica japonica* Lützen and Takahashi, 2003), and holothurians (*e.g.*, *Austrodevonia sharnae* Middelfart and Craig, 2004). Another 152 species (40%) in the dataset are not restricted in habitat or in distribution within the context of the original author(s).

This dataset also includes new species from a wide range of water depths. Okutani and Soh (2005: 28) stated that "bathymetrical difference is another important clue for speciation," and several authors included in this survey (Allen 2000, 2007, Sasaki and Haga 2007) stated depth criteria in support of new species (*e.g.*, the new species in question is the first in a given group from the intertidal zone or from abyssal depths). Minimum recorded depth shows a mean of 444.1 ± 841.41 m (N = 327 m, ranging 0-7,299 m, with intertidal scored as 0), with 138 species (of 327 or 42%) from >100 m (Fig. 2). Maximum recorded depth showed a mean of 733.14 ± 884.46 m (N = 324 spp., ranging 0-7,333 m, with intertidal scored as 0), but included substantially more species (222 of 327 species, or 68%) from >100 m. The range of depths recorded for new species (*i.e.*, the difference between maximum and minimum recorded depth; scored for 324 spp.) ranges from 0-6,795 m, with 146 species (of 324 or 45%) from >100 m and 21 species (of 324 or 6%) from >1,000 m. So, we can reasonably summarize that we are currently describing many new species from deep water, another inadequately explored habitat, but that new shallow-water species also continue to be discovered.

Summary and discussion

What do these 381 new species descriptions reflect about speciation? At first glance, not much. Malacologists are clearly still exploring and continuing to document undiscovered diversity on a regular basis. So, these new species are not newly isolated species, but rather of the results of intensified collecting (*e.g.*, MUSORSTOM Project, http://www.mnhn.fr/musorstom/index.html). This small sample of the recent literature includes the first lucinid from hydrothermal vents (*Bathyaustriella thionipta* Glover

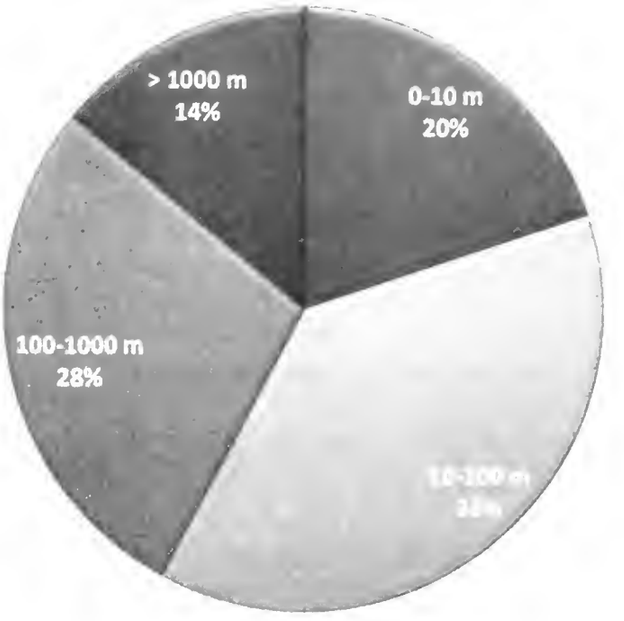

Figure 2. Minimum recorded depth (N = 327 m, ranging from 0 to 7,299 m, with intertidal scored as 0) in new marine bivalve species described during 2000-2009. 42% of the species inhabit deep water (>100 m).

et al., 2004), the first deep-water neoleptonid (*Neolepton profundorum* Allen, 2000), the first abyssal *Epilepton* (*E. elpis* Allen, 2007), the first galeommatoidean associated with Australian holothurians (*Austrodevonia sharnae* Middelfart and Craig, 2004), the smallest known bathymodioline (*Bathymodiolus taiwanensis* Cosel, 2008b), the second largest living mytilid (*Gigantidas gladius* Cosel and Marshall, 2003), and the first ostreid to be described genetically (*Crassostrea hongkongensis* Lam and Morton, 2003). This ten-year record includes large and small species over a wide taxonomic and geographic range. Their descriptions rely heavily upon shell morphology (most often sculpture, shape, and hinge), but include substantial contributions from soft anatomy and minor contributions from molecules and phylogenetic analyses. Amano and Lutaenko (2004: 19) noted that their new species, *Linopsis oliveri*, has no fossil record and "might have arisen very recently"; this statement could likely be supposed of many of these recently described species.

In the apparent paucity of allopatric species barriers in the marine realm, one important key to apparently sympatric speciation is, according to this study, specialization. Marine gastropods illustrate this well, with their many dietary and developmental (especially nonplanktonic) strategies (reviewed by Krug 2011). Because the vast majority of bivalves are broadcast-spawning filter feeders, such cases are rare (although not unheard of, see below). However, there are other ways that marine bivalves can specialize, and a number of them are present in this dataset. First and most generally, the high

percentages of deep-water and otherwise restricted bivalve species suggest that ecological or physiological limits can set up barriers to gene flow. Jones *et al.* (2005) convincingly provided molecular phylogenetic evidence for multiple invasions of deep-water hydrocarbon seep and hydrothermal vent habitats by species of Bathymodiolinae (Mytilidae), showing the effectiveness of bathymetric specialization for the radiation of this subfamily.

In one of the few examples of dietary specialization in bivalves, Järnegren *et al.* (2007) showed their new species of file clam, *Acesta oophaga* Järnegren, Schander, and Young, 2007, to be a highly specialized member of the cold seep community in the northern Gulf of Mexico. Not only is it restricted in habitat, it is unique among bivalves, and so far among all marine animals, in its specialized method of feeding. This species is always found byssally attached to the sabellid polychaete *Lamellibrauchia luymesi* and ingests the eggs ejected by the tubeworm, capitalizing on the chemosynthetic abilities of its prey to survive in a reducing environment without the cost of maintaining symbionts of its own. Prior to this study and the direct observations by manned submersible that it included, *A. oophaga* was misidentified in collections as *A. bullisi* Vokes, 1963 or *A. excavata* (Fabricius, 1779), two species found in the general distributional range of *A. oophaga*, but neither of which are associated with either seeps or tubeworms. Because exploration of the deep sea, and discovery of additional new deep-sea species, are strongly represented in this dataset and can be expected to continue in the immediate future, we can expect to see additional research along the lines of these last two examples, which provide at least circumstantial support for specialization as a component of possible mechanisms of bivalve speciation.

Another type of specialization is that of host associations. In addition to the bivalve species mentioned above that are commensal with other invertebrates, Richter *et al.* (2008) showed that *Tridacna* spp. (including the new species *T. costata* Roa-Quiaoit, Kochzius, Jantzen, Zibdah, and Richter, in Richter *et al.*, 2008), like some species of coral, differ in the various strains or clades of zooxanthellae (*Symbiodinium*) found in their mantle tissues and on which they physiologically depend. These differences in symbiont composition are correlated with habitat, with those harboring clade A symbionts being found on reef flats, and those hosting both clades A and B inhabiting a wider depth range, but importantly, able to invade deeper fore reefs. Such differences have apparently prompted adaptation among *Tridacna* spp. to particular light regimes associated with their individual complements of phototrophic symbionts. Such a case might have resulted from a cline within a continuous population (as discussed, *e.g.*, by Valentine and Jablonski 1983).

Species complexes

Although not clearly evident from recent new species descriptions, examples suggesting ongoing speciation in the form of cryptic species complexes can be found in the marine bivalve record. Species complexes are either (1) groups of closely related nominal species whose morphological boundaries are difficult to define (AKA sibling, incipient, or cryptic species; Knowlton 1993, Gardner 1994, 1996), or (2) highly variable species that have been revealed by molecular or other analyses to be composed of more than one species, but whose morphological boundaries remain elusive (Goffredi *et al.* 2003, Lee and Ó Foighil 2004). Here are brief descriptions of seven examples that have appeared in recent literature:

(1) The species *Mytilus edulis* Linnaeus, 1758 [native to northeastern and northwestern Atlantic], *M. galloprovincialis* Lamarck, 1819 [northeastern Atlantic], *M. trossulus* Gould, 1850 [northeastern and northwestern Pacific, northeastern and northwestern Atlantic], *M. californianus* Conrad, 1837 [northeastern Pacific], and *M. chilensis* (Hupé, 1854) [southeastern Pacific] comprise a species complex of superficially similar "blue mussels" in temperate intertidal habitats around the globe. They byssally attach to hard substrata and are broadcast spawners. They have been harvested for food by humans for at least the past 12,000 years (Erlandson *et al.* 2008). Some of these species are additionally raised in aquaculture and are (importantly) anthropogenically transported alive from market to market, further blurring species boundaries. For example, two species pairs (*M. edulis-galloprovincialis* in southwestern Europe and *M. edulis-trossulus* in Scandinavia and Atlantic Canada) are known to hybridize and have become models for studies of hybrid-zone structure, involving considerations such as genetic identification methods, local adaptation, hybrid fitness, asynchronous spawning, gamete recognition, and fertilization preference (*e.g.*, Gardner 1994, 1996, Rawson *et al.* 1996, Comesaña *et al.* 1999, Bierne *et al.* 2002a, 2002b, Riginos and Cunnngham 2005, Toro *et al.* 2005, Braby and Somero 2006). Although their genotypes are clearly identified, little morphological resolution is available and they comprise a classic example of a species complex. No resolution of the taxonomic issues, either through synonymy or erection of new species, has been attempted.

(2) The small-bodied pearl oysters of the world—*Pinctada imbricata* Röding, 1798 [western Atlantic], *P. fucata* (Gould, 1850) [Indo-West Pacific], *P. martensii* (Dunker, 1857) [Japan], *P. radiata* (Leach, 1814) [Persian Gulf]—are widely raised in aquaculture or harvested for use in pearl culture. These names are historically regional, and in at least one case, political, with strong ties to the commercial pearl industry; Japanese perliculturists prefer to consider their pearl oyster, which they call *P. martensii*, endemic to Japan.

Biologically, however, according to recent molecular data and phylogenetic analyses (Masaoka and Kobayashi 2002, 2003, 2005a, 2005b, Yu and Chu 2006, Tëmkin 2007), all seem to belong to one circumglobal species complex "characterized by substantial intraspecific variation largely due to color and size differences" (Tëmkin 2007: 318). No morphological characters have been found to dispute the molecular results and the four species have been synonymized under the senior synonym *P. imbricata*.

(3) Members of the galeommatoidean genus *Lasaea* [*e.g.*, *L. adansoni* (Gmelin, 1791), *L. rubra* (Montagu, 1803), *L. maoria* (Powell, 1933), *L. subviridis* Dall, 1899, and others] inhabit intertidal turf-algal and rock-crevice habitats worldwide. Unusually for marine bivalves, they are hermaphroditic brooders with direct development. Their complex genetics includes instances of polyploidy and within-island cladogenesis associated with their specialized larval development, and proposed dispersal/colonization events are equally complex (Ó Foighil and Smith 1996, Ó Foighil and Jozefowicz 1999, Ó Foighil and Thiriot-Quiévreux 1999, Park and Ó Foighil 2000, Ó Foighil *et al.* 2001). Although hybridization apparently does not occur, sympatric asexual clones have been detected (Crisp and Standen 1988, Ó Foighil and Smith 1995) that "represent highly divergent phylogenetic lineages" (Ó Foighil *et al.* 2001). Morphological differences within this species complex are unresolved and all species names continue to be used as distinct.

(4) Small mytilids of the genus *Brachidontes* are byssate on shallow-water rocks and have planktonic larvae. They are tolerant of high salinities and temperatures, and show high levels of morphological variability. A molecular phylogeny of the species *B. variabilis* (Krauss, 1848) from the Mediterranean Sea, Red Sea [from these last two often as *B. pharaonis* (P. Fischer, 1870)], Indian Ocean, and western Pacific indicated three cryptic species from these locations, diverging in the Miocene Epoch, approx. 6-11 million years ago (Terranova *et al.* 2007). Vicariance events during Pleistocene glaciations and the opening of the Red Sea were proposed as the mechanisms underlying speciation in this complex although morphological characters supporting this separation are as yet unclear. A similar study of *B. exustus* (Linnaeus, 1758) in the western Atlantic revealed a species complex of five as-yet-unnamed distinct genotypes in peninsular Florida and the Caribbean alone, likely driven in this case by post-recruitment ecological barriers (Lee and Ó Foighil 2004, 2005).

(5) The file clams *Limatula ovalis* Thiele, 1912 and *L. pygmaea* (Philippi, 1845) inhabit deep waters of the Antarctic or sub-Antarctic regions, respectively, on soft bottom in cobweb-like nests of their own construction. Shells of *Limatula* spp. are well-known to be highly conservative (*i.e.*, closely similar), making shell characters difficult to use for species identification (independent of locality) or phylogenetic reconstruction. A recent molecular phylogeny (Page 2002, Page and Linse 2002) supports their divergence less than 20 million years ago. In this case, the Antarctic Polar Front has been proposed as a temperature barrier maintaining the two species.

(6) Vesicomyid clams are dominant organisms in the world's chemosynthetic-based deep-sea communities. Species-level variation in the ability to uptake product from sulfide-metabolizing symbionts suggests that physiological specialization is at work in maintaining sympatric species (Goffredi and Barry 2002). In another study involving molecular and morphometric analyses (Goffredi *et al.* 2003), five lineages—*Vesicomya pacifica* (Dall, 1891), *V. lepta* (Dall, 1886), and three undescribed species of *Vesicomya*—were identified. All five species are broadcast spawners, which are nevertheless segregated by depth and thus presumably diverged and are maintained by bathymetric and substratum factors exerted at the recruitment level. Although two existing species names have been applied to two of these lineages, morphological criteria distinguishing these plus the three undescribed species remain unconfirmed.

(7) The western Atlantic oyster *Crassostrea virginica* (Gmelin, 1791) ranges in hyposaline estuaries from eastern Canada to the Gulf of Mexico, and is well-known to include physiological "races" (Ahmed 1975, Dittman *et al.* 1998). Morphological evidence first provided in the 1950s (Loosanoff and Nomejko 1951) is complicated by the fact that morphological characters are extremely phenotypically plastic in Ostreidae, a taxonomic difficulty among bivalves that is certainly not unique to this family. Indeed, *C. hongkongensis* Lam and Morton, 2003 went unrecognized in part because morphology is so variable among oysters. Morphological data for *C. virginica* have since been supplemented by molecular data (*e.g.*, Peterson 2006, Varney *et al.* 2009) that confirm clines even in small parts of the geographic range, yet accepted nomenclature continues to recognize a single, variable species. This case is complicated by the fact that *C. virginica* is widely cultivated in aquaculture in the region for human consumption. Other species of oysters (*e.g.*, *Ostrea edulis* Linnaeus, 1758 of Europe; Ahmed 1975) show similar patterns.

Table 2 summarizes these seven examples, showing that species complexes can be either revealed or resolved by molecular analyses, even in the absence of morphological evidence. In two of these species complexes (*Brachidontes* and *Vesicomya*), incipient speciation is clearly suggested; alternatively, we simply could be at a stage of correcting overly conservative taxonomy. More importantly, this kind of research on species complexes has revealed apparent barriers to gene flow in the marine realm that are physiological (sympatric, *e.g.*, temperature in *Limatula*, bathymetry in

Table 2. Species complexes discussed herein, with their initial level of variation, support from molecular and morphological data, presumed reproductive barriers, the beginning and ending numbers of species, and whether molecular data revealed or resolved species complex issues. See text for pertinent references.

Taxa	Variation	Molecules	Morphology	Barrier(s)	Species	Outcome
Mytilus	low	yes	no	???	5 → (5)	revealed
Pinctada	low	no	no	none	4 → 1	resolved
Lasaea	low	yes	no	isolated "islands"	n → ?	revealed
Brachidontes	high	yes	no	Miocene vicariance or salinity/ temperature	1 → 3-4	revealed
Limatula	low	yes	yes	temperature	2 → 2	resolved
Vesicomya	low	yes	no	bathymetry, substratum	2 → 5	revealed
Crassostrea	high	yes	yes	???	1 → ?	revealed

Vesicomya) or physical (allopatric, *e.g.*, Miocene vicariance events in *Brachidontes,* isolated "island" habitats in *Lasaea*), some of which can be, nevertheless, broken down by anthropogenic transport (*e.g.*, resulting in hybridization in *Mytilus*).

Phylogenetic analyses

Phylogenetic analyses, especially at low (generic or familial) taxonomic levels, can show clade patterns that reveal radiation and suggest the innovations that facilitated that radiation. One must look for either of two patterns: (1) speciose clades as part of strongly disparately sized sister taxa (here size indicating numbers of species/clade, *i.e.*, indicating radiation of the more species-rich sister) coupled with the synapomorphies (*i.e.*, the recognized evolutionary innovations) of the more species-rich sister, or (2) unresolved polytomies that might indicate unacknowledged species complexes or rapid ongoing speciation. The following text provides one example of each pattern.

When numbers of recognized species per superfamily are superimposed on a bivalve tree (Fig. 3), four sister pairs are revealed showing highly unequal species per sister and recognized synapomorphies suggesting innovations: (1) Unionoidea (688 spp., all freshwater) and Trigonioidea (5 spp.) suggest the glochidium (*i.e.*, the specialized unionoidean larval type that depends upon a fish host to complete metamorphosis) as the innovation promoting unionoidean radiation; (2) Lucinoidea (500 spp.) and Galeommatoidea (30 spp.) + Pholadoidea (170 spp.) suggest commensalism with sulfide-oxidizing bacteria and mucus-tube feeding that allow habitation of a reducing environment as innovations of the more species-rich sister; (3) Tellinoidea (645 spp.) and Chamoidea (70 spp.) + Cardioidea (255 spp.) suggest that the cruciform muscle and very long siphons are innovations of Tellinoidea that allowed very deep burrowing in soft sediments; and (4) Veneroidea (820 spp.) and Arcticoidea (1 spp.) suggest that the aortic bulb (a muscular, spongy structure on the ventral side of the posterior aorta, which prevents rupture of the heart when the siphons and foot retract suddenly) is an innovation that facilitates rapid retraction and valve closure in siphonate veneroideans. According to the dates of first occurrence in the fossil record, these innovations occurred in the Triassic (1), Ordovician (2), and Jurassic (3, 4), in strong congruence with the cladogram topology.

The molecular phylogeny of Lucinoidea presented by Williams *et al.* (2004) shows the family Lucinidae as a strongly supported polytomy, with one of its clades, called "Lucinid clade B + *Phacoides,*" also as a strongly supported polytomy. This tree was generated by molecular data alone, so morphological synapomorphies are difficult to identify; however, the authors did accept that some characters that were previously considered diagnostic for one group or another were homoplastic according to this tree. All lucinids to date are known to harbor ctenidial chemosynthetic bacteria within bacteriocytes. Chemosymbiosis allows lucinids to inhabit oligotrophic environments, which is not unique, but this specialized location for the symbionts differs from that of other symbiont-bearing bivalves, and might be a more recent novelty than can be estimated from the fossil record. Given the well-studied status of this family, the polytomies in this analysis likely do not indicate unrecognized species complexes, but rather possible parts of the cladogram with taxa undergoing rapid evolutionary change. This suggests a focus for fruitful future research.

CONCLUSIONS

The three types of data from the recent literature presented here—new species descriptions, species complexes, and phylogenies at low taxonomic levels—provide insight into possible mechanisms of speciation occurring in recent marine bivalves. Specialization is revealed as one of the more important factors facilitating speciation in bivalves in the seemingly homogenous sea. Ecological or physiological specialization can create effective barriers between sympatric species (a documented phenomenon in animals such as fish, aphids, and gastropods, *e.g.*, Hollander *et al.* 2005), and is illustrated here by bathymodioline mussels invading deep-water

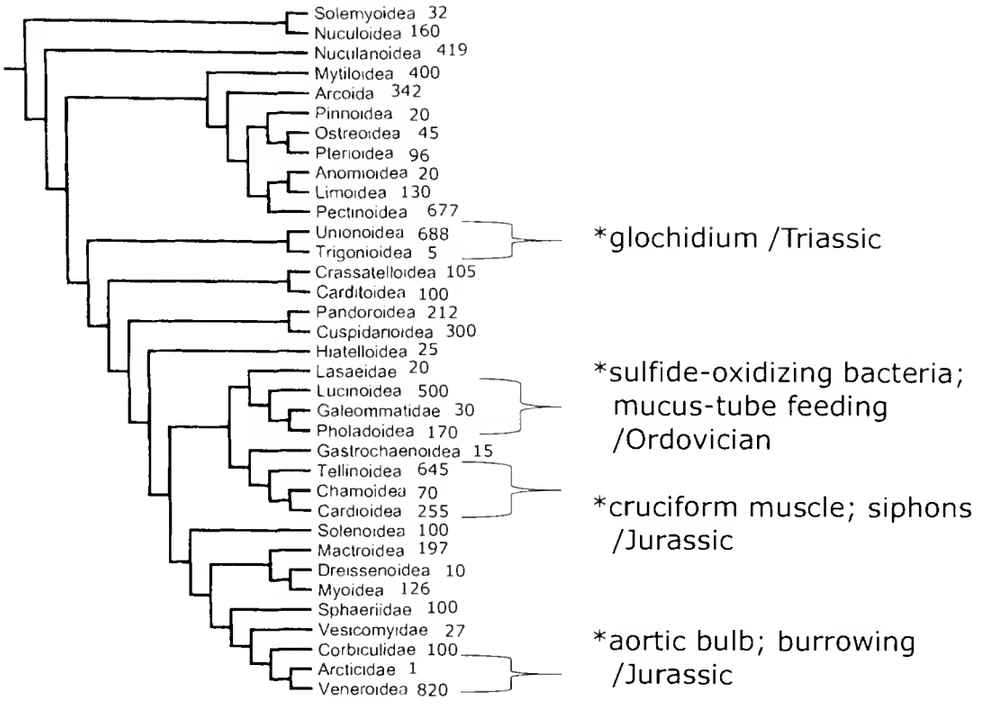

Figure 3. The numbers of recognized species per Recent bivalve superfamily (from Mikkelsen and Bieler 2008) superimposed on the morphological + molecular bivalve cladogram by Giribet and Wheeler (2002: fig. 11). Four disparately sized sister pairs are recognized (Unionoidea-Trigonoidea; Lucinoidea-Galeommaoidea + Pholadoidea; Tellinoidea-Chamoidea + Cardioidea; Veneroidea-Arcticoidea), with their synapomorphies (*i.e.*, innovations that drove radiation of the more species-rich sister) and dates of first occurrence from the fossil record.

habitats, an egg-eating *Acesta*, multiple strains of zooxanthellae segregating *Tridacna* spp. by depth, the Antarctic Polar Front as a temperature barrier for *Limatula* spp., larval vesicomyid clams responding to bathymetric and substratum factors, and physiological "races" of oysters. This analysis also identified two probable cases of allopatric speciation: direct-developing *Lasaea* spp. in isolated turf or crevice "islands," and *Brachidontes* diverging in response to Miocene vicariance events. It does not follow, however, that species-rich clades are necessarily more "specialized" bivalves overall; few bivalve experts would call members of the Veneroidea "specialized," yet the aortic bulb apparently allowed them a physiological advantage over other members of their ancestral clade. Together these data defeat the concept of a Marine Speciation Paradox in bivalves—speciation clues are merely more subtle than those in terrestrial systems and most often act at the physiological, rather than physical, level.

One factor that could not be traced by this analysis but which could impact the process (or at least the rate) of speciation in bivalves is life span. Certainly, researchers use the fruit fly (*Drosophila melanogaster*) as a model in evolutionary studies in part because its extremely short life span (30 days or less) allows one to observe rapid changes. One of the largest-bodied new species tabulated here is *Neopycnodonte zibrowii* Gofas, Salas, and Taviani, in Wisshak *et al.*, 2009; the authors claimed that through radiocarbon dating, they determined that this bivalve can reach >500 years of age (at a maximum size of 300 mm). Species that are raised in aquaculture have generally well-understood life spans; others have been analyzed via the growth increments in their shells. A few examples are: *Donax* spp., 1 year (Buttemer *et al.* 2010), *Argopecten gibbus* (Linnaeus, 1758), 2 years (Roe *et al.* 1971), *Pinctada margaritifera* (Linnaeus, 1758), 6 years (in culture; Landman *et al.* 2001), *Mytilus edulis*, 18 years (Sukhotin *et al.* 2007), *Mya arenaria* Linnaeus, 1758, 28 years, *Spisula solidissima* (Dillwyn, 1817), 30 years, *Mercenaria mercenaria* (Linnaeus, 1758), 75 years (the last three summarized by Jones 1989), *Tridacna gigas* (Linnaeus, 1758), 100 years (Comfort 1957), *Panopea generosa* Gould, 1850, 120 years (B. Black, http://people.oregonstate.edu/~Blackbry/reserach.htm), and *Arctica islandica* (Linnaeus, 1767), 410 years (Wanamaker *et al.* 2008). Does a long life span impede speciation in any way (*e.g.*, long-lived Arcticoidea with 1 species versus shorter-lived Veneroidea with 820 species)? Additional consideration of this topic is warranted.

To further interpret this record, we need (1) a robust phylogenetic framework for Bivalvia [currently being pursued by the BivAToL Project (www.bivatol.org) and other groups], (2) additional molecular work on taxa with "difficult" (*i.e.*, variable or homogenous) morphology, in search of unrecognized species complexes, especially where suggested by wide geographical or depth distributions [two such examples are *Hiatella arctica* (Linnaeus, 1767), which ranges from Greenland to Mexico, and worldwide species of *Perna* that as commercial "green mussels" have been anthropogenically introduced to many non-native locations], and (3) additional taxon-rich genus-level phylogenies in which to identify novelties and rapidly evolving clades.

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Appendix. New marine bivalve species and subspecies (arranged alphabetically by family) described during the years 2000-2009. Tabulated data are taken from the original descriptions. Y, yes; N, no; na, not applicable or none listed; see footnotes for other abbreviations.

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Acar marsupialis Oliver and Holmes, 2004	Arcidae	IO	17	2.58	N	N	N	N	Y	na
Acar transmar Simone, 2009b	Arcidae	WA	8	5	Y	Yd	N	P	N	na
Arca koumaci Lutaenko and Maestrati, 2007	Arcidae	IP	57	6.1	N	N	N	N	N	NC
Barbatia diphaeonotus Oliver and Holmes, 2004	Arcidae	IO	18	6.55	Y	N	N	N	N	na
Barbatia pyrrhotus Oliver and Holmes, 2004	Arcidae	IO	18	4.9	Y	N	N	N	N	na
Samacar (Pseudoporterius) aleutica Kamanev, 2007	Arcidae	EP	168	18	N	N	N	N	Y	NC
Samacar (Samacar) kurilensis Kamanev, 2007	Arcidae	NWP	368	20.9	Y	Y	N	N	Y	na
Astarte anholti Petersen, 2001	Astartidae	EA	22	24.8	N	N	N	N	Y	NC
Astarte belti Petersen, 2001	Astartidae	EA	15	35	N	N	N	N	Y	NC
Astarte bornholmi Petersen, 2001	Astartidae	EA	93	22.7	Y	N	N	N	Y	NC
Astarte elonga Petersen, 2001	Astartidae	AC	na	23.5	N	N	N	N	Y	NC
Astarte falsteri Petersen, 2001	Astartidae	EA	70	23	N	N	N	N	Y	NC
Astarte fjordi Petersen, 2001	Astartidae	EA	24	19	N	N	N	N	Y	NC
Astarte jenseni Petersen, 2001	Astartidae	EA	30	36.3	N	N	N	N	Y	NC
Astarte klinti Petersen, 2001	Astartidae	EA	38	15.5	N	N	N	N	Y	NC
Astarte moerchi Petersen, 2001	Astartidae	AC	na	37.9	N	N	N	N	Y	NC
Astarte neocrassa Petersen, 2001	Astartidae	AC	na	27.2	N	N	N	N	Y	NC
Astarte nordi Petersen, 2001	Astartidae	EA	56	33.5	N	N	N	N	Y	NC
Astarte nuuki Petersen, 2001	Astartidae	AC	na	38	N	N	N	N	Y	NC
Astarte silki Petersen, 2001	Astartidae	EA	93	27.5	Y	N	N	N	Y	NC
Astarte vaigati Petersen, 2001	Astartidae	AC	na	30	N	N	N	N	Y	NC
Acrosterigma capricorne Vidal and Kirkendale, 2007	Cardiidae	IP	1350	58.1	Y	N	N	N	Y	na
Acrosterigma suduirauti Vidal and ter Poorten, 2007	Cardiidae	IP	150	37.8	N	N	N	N	Y	na
Cardium maxicostatum ter Poorten, 2007	Cardiidae	EA	28	130	N	N	N	N	N	na
Ctenocardia fijianum Vidal and Kirkendale, 2007	Cardiidae	IP	34	12	N	N	N	N	Y	na
Ctenocardia gustavi Vidal and Kirkendale, 2007	Cardiidae	IP	54	20	Y	N	N	N	N	na
Ctenocardia subfestivum Vidal and Kirkendale, 2007	Cardiidae	IP, NWP	400	10.3	Y	N	N	N	N	na
Fragum grasi ter Poorten, 2009	Cardiidae	IP	21	8	Y	N	N	N	N	NC
Frigidocardium helios ter Poorten and Poutiers in ter Poorten, 2009	Cardiidae	IP	102	9.5	Y	N	N	N	N	NC
Frigidocardium iris Huber and ter Poorten, 2007	Cardiidae	IP	200	28.3	N	N	N	N	N	na
Frigidocardium sancticaroli ter Porten and Poutiers in ter Poorten, 2009	Cardiidae	IP	210	11.3	Y	N	N	N	N	NC
Frigidocardium valdentatum Poutiers, 2006	Cardiidae	IP	460	27.5	N	N	N	N	N	NC

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Fulvia colorata Vidal and Kirkendale, 2007	Cardiidae	IP	82	16.4	Y	Y	N	N	N	na
Fulvia imperfecta Vidal and Kirkendale, 2007	Cardiidae	IP	53	10.9	N	N	N	N	N	na
Fulvia subquadrata Vidal and Kirkendale, 2007	Cardiidae	IP	286	17.2	N	N	N	N	N	na
Fulvia vepris Vidal and Kirkendale, 2007	Cardiidae	IP	na	15.8	N	N	N	N	Y	na
Lunulicardia orlini Mienis, 2009	Cardiidae	EA	49	44.59	N	N	N	N	N	na
Lyrocardium anaxium dekkeri Mienis, 2009	Cardiidae	EA	92	55.62	Y	Y	N	N	N	na
Microcardium trapezoidale Poutiers, 2006	Cardiidae	IP	806	20	Y	N	N	N	N	NC
Microcardium velatum ter Poorten and Poutiers in ter Poorten, 2009	Cardiidae	IP	356	16	Y	N	N	N	Y	NC
Parvicardium carrozzai Van Aartsen and Goud, 2001	Cardiidae	EA	300	9	N	N	N	N	N	na
Pseudofulvia arago Vidal and Kirkendale, 2007	Cardiidae	IP	107	27	Y	Yd	N	N	Y	na
Pseudofulvia caledonica Vidal and Kirkendale, 2007	Cardiidae	IP	446	13.4	Y	Yd	N	N	N	na
Tridacna costata Roa-Quiaoit, Kochzius, Jantzen, Zibdah, and Richter in Richter *et al.*, 2008	Cardiidae	IO	2	320	Y	Yd	Y	P	Y	C
Trifaricardium morrisoni ter Poorten and Huber, 2007	Cardiidae	IO	300	11.9	N	N	N	N	N	na
Vasticardium lomboke Vidal, 2003	Cardiidae	IP	sh	28.9	N	N	N	N	Y	na
Vasticardium subassimile Vidal, 2003	Cardiidae	IP	na	65.4	N	N	N	N	N	na
Vepricardium alboliamatum Hylleberg and Vidal in Vidal, 2000	Cardiidae	IP	na	44.2	N	N	N	N	Y	R
Vepricardium vidali ter Poorten and Dekker, 2002	Cardiidae	IO	sh	54.3	Y	Y	N	N	Y	na
Chama cerion Matsukuma, Paulay, and Hamada, 2003	Chamidae	IP,IO, NWP	30	25.9	Y	N	N	N	N	na
Chlamydoconcha avalvis Simone, 2008	Chlamydoconchidae	WA	6	15	Y	Yd	N	N	Y	na
Bryopa aligamenta Morton, 2005	Clavagellidae	NWP	na	20	Y	Y	N	N	Y	na
Dianadema mascarensis Oliver and Holmes, 2004	Clavagellidae	IO	17	9.48	N	N	N	N	Y, H	na
Austrocardiella pouli Middelfart, 2002a	Condylocardiidae	IO	150	0.86	N	N	N	N	E	R
Benthocardiella burtonae Middelfart, 2002a	Condylocardiidae	IP	73	1.42	N	N	N	N	Y	R
Benthocardiella darwinensis Middelfart, 2002a	Condylocardiidae	IP	na	1.21	N	N	N	N	Y	R
Carditella marieta Coan, 2003	Condylocardiidae	EP	46	2	N	N	N	N	N	R
Condylocardia cometa Middelfart, 2002a	Condylocardiidae	IP	131	1.35	N	N	N	N	Y	R
Condylocardia elongata Coan, 2003	Condylocardiidae	EP	30	2.4	N	N	N	N	Y	R
Condylocardia fernandina Coan, 2003	Condylocardiidae	EP	110	2.1	N	N	N	N	Y	R

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Condylocardia galapagana Coan, 2003	Condylocardiidae	EP	228	2.6	N	N	N	N	N	R
Condylocardia geigeri Coan, 2003	Condylocardiidae	EP	124	1.9	N	N	N	N	Y	R
Condylocardia kaiserae Coan, 2003	Condylocardiidae	EP	274	3.1	N	N	N	N	N	R
Condylocardia koosae Coan, 2003	Condylocardiidae	EP	274	2.2	N	N	N	N	Y	R
Condylocardia sparsa Coan, 2003	Condylocardiidae	EP	35	2.2	N	N	N	N	N	R
Condylocuna annieae Middelfart, 2002a	Condylocardiidae	IP	201	1.15	N	N	N	N	Y	R
Condylocuna jimbecki Middelfart, 2002a	Condylocardiidae	IP	201	0.98	N	N	N	N	Y	R
Condylocuna tricosa Middelfart, 2002a	Condylocardiidae	IP	103	0.88	N	N	N	N	N	R
Crassacuna crassisculpta Middelfart, 2002b	Condylocardiidae	IP	124	2.13	N	N	N	N	N	R
Cuna deltoides Middelfart, 2002b	Condylocardiidae	IP	81	1	N	N	N	N	N	R
Cuna libbyae Middelfart, 2002b	Condylocardiidae	IP	1465	1.9	N	N	N	N	N	R
Cuna microcentrica Middelfart, 2002b	Condylocardiidae	IP	81	1.3	N	N	N	N	N	R
Cuna navicula Middelfart, 2002b	Condylocardiidae	IP	128	1.8	N	N	N	N	N	R
Cuna ramus Middelfart, 2002b	Condylocardiidae	IP	201	2.7	N	N	N	N	N	R
Isodontocardia microcardia Middelfart, 2002a	Condylocardiidae	IP	65	1.58	N	N	N	N	N	R
Minicuna cuniformis Middelfart, 2002b	Condylocardiidae	IP	31	1.08	N	N	N	N	Y	R
Warrana brucemarshalli Middelfart, 2002b	Condylocardiidae	IP	36	2.13	N	N	N	N	N	R
Warrana flexuosa Middelfart, 2002b	Condylocardiidae	IP	150	1.83	N	N	N	N	N	R
Warrana limata Middelfart, 2002b	Condylocardiidae	IP	294	2.82	N	N	N	N	N	R
Warrana pauciconcentrica Middelfart, 2002b	Condylocardiidae	IP	85	1.01	N	N	N	N	N	R
Warrana pellucida Middelfart, 2002b	Condylocardiidae	IP	256	2.07	N	N	N	N	N	R
Warrana punicea Middelfart, 2002b	Condylocardiidae	IP	155	1.47	N	N	N	N	N	R
Warrana triangulata Middelfart, 2002b	Condylocardiidae	IP	23	0.9	N	N	N	N	Y	R
Warrana westralis Middelfart, 2002b	Condylocardiidae	IP	158	1.26	N	N	N	N	N	R
Westaustrocuna albanyensis Middelfart, 2002b	Condylocardiidae	IP	146	1.05	N	N	N	N	N	R
Westaustrocuna keegani Middelfart, 2002b	Condylocardiidae	IP	155	1.14	N	N	N	N	N	R
Corbula colimensis Coan, 2002b	Corbulidae	EP	112	14	N	N	N	N	N	na
Corbula grovesi Coan, 2002b	Corbulidae	EP	732	11	N	N	N	N	Y	na
Corbula otra Coan, 2002b	Corbulidae	EP	55	26.1	N	N	N	N	N	na
Corbula tarasconii Arruda, Domaneschi, Francisco, and de Barros, 2007	Corbulidae	WA	65	7.2	N	N	N	N	N	na
Crassatina rikai Lamprell, 2003	Crassatellidae	IP	300	14.7	N	N	N	N	Y	R
Crassatina suduirauti Lamprell, 2003	Crassatellidae	IP	180	26	N	N	N	N	N	R
Cuspidaria altenai Knudsen, 2005	Cuspidariidae	WA	300	12.4	N	N	N	N	Y	NC
Cuspidaria luymesi Knudsen, 2005	Cuspidariidae	WA	94	15.9	Y	Y	N	N	Y	NC
Plectodon lepidus Marshall, 2002	Cuspidariidae	IP	83	6.1	Y	N	N	N	N	na
Plectodon pruniosus Marshall, 2002	Cuspidariidae	IP	710	6.25	N	N	N	N	N	na
Plectodon regalis Marshall, 2002	Cuspidariidae	IP	805	6.1	Y	N	N	N	Y	na

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Pseudogrippina wangellanica Marshall, 2002	Cuspidariidae	IP	437	2.8	Y	N	N	N	Y	na
Rhinoclama brooki Marshall, 2002	Cuspidariidae	IP	274	6.6	N	N	N	N	Y	na
Rhinoclama tangaroa Marshall, 2002	Cuspidariidae	IP	437	2.52	Y	N	N	N	N	na
Centrocardita pileolata Oliver and Holmes, 2004	Cyamiidae	IO	18	4	Y	N	N	N	Y	na
"Lutetina" capricornica Oliver and Holmes, 2004	Cyamiidae	IO	17	2	N	N	N	N	Y	na
Pseudokellia franki Zelaya and Ituarte, 2009	Cyamiidae	AN	100	4.2	Y	Yd	N	N	Y	NC
Peregrinamor gastrocluaenans Kato and Itani, 2000	Gaimardiidae	NWP	int	6.4	Y	Yd	N	N	Y, H	na
Austrodevonia sharnae Middelfart and Craig, 2004	Galeommatidae	IP	int	4.4	Y	Y	N	N	Y, H	na
Duoconclavis piscator Middelfart, 2005	Galeommatidae	IP	int	5.2	Y	Yd	N	N	Y	R
Ephippodontomorpha hirsutus Middelfart, 2005	Galeommatidae	IP	na	9.9	Y	Yd	N	N	H	R
Galeomma phuketi Lützen and Nielsen, 2005	Galeommatidae	IP	int	6	Y	Y	N	N	Y	NC
Galeomma sagenata Oliver and Holmes, 2004	Galeommatidae	IO	18	4.81	Y	Y	N	N	Y	na
Nudiscintilla glabra Lützen and Nielsen, 2005	Galeommatidae	IP	int	6.8	Y	Yd	N	N	Y	NC
Parabornia palliopapillata Simone, 2001	Galeommatidae	WA	sh	10	Y	Yd	N	N	H	na
Scintilla macrodactylus Lützen and Nielsen, 2005	Galeommatidae	IP	sh	4.6	Y	Yd	N	N	Y	NC
Scintilla minor Lützen and Nielsen, 2005	Galeommatidae	IP	sh	4.3	Y	Y	N	N	Y	NC
Scintilla mortoni Lützen and Nielsen, 2005	Galeommatidae	IP	sh	5.3	Y	Yd	N	N	Y	NC
Scintilla ovalis Lützen and Nielsen, 2005	Galeommatidae	IP	sh	8.2	Y	Yd	N	N	Y	NC
Scintilla papillosa Lützen and Nielsen, 2005	Galeommatidae	IP	int	9.8	Y	Yd	N	N	Y	NC
Scintilla sannio Lützen and Nielsen, 2005	Galeommatidae	IP	sh	10.6	Y	Yd	N	N	Y	NC
Scintilla siamense Lützen and Nielsen, 2005	Galeommatidae	IP	na	11	Y	Yd	N	N	Y	NC
Scintilla unicornia Lützen and Nielsen, 2005	Galeommatidae	IP	sh	7.4	Y	Yd	N	N	Y	NC
Scintilla verrucosa Lützen and Nielsen, 2005	Galeommatidae	IP	int	6.8	Y	Yd	N	N	Y	NC
Troglodytoconcha carpentariensis Middelfart, 2005	Galeommatidae	IP	11	7.5	Y	Yd	N	N	Y, H	R
Aclistothyra orientalis Lützen and Nielsen, 2005	Galeommatidae	IP	na	10	Y	Yd	N	N	Y	NC
Scintilla agilis Lützen and Nielsen, 2005	Galeommatidae	IP	sh	6.1	Y	Yd	N	N	Y	NC
Scintilla larcombae Oliver and Holmes, 2004	Galeommatidae	IO	18	4	Y	Y	N	N	Y	na

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Scintilla longitentaculata Lützen and Nielsen, 2005	Galeommatidae	IP	int	5.5	Y	Yd	N	N	Y	NC
Scintilla lynchae Oliver and Holmes, 2004	Galeommatidae	IO	1	10	Y	Y	N	N	Y	na
Glycymeris (Glycymeris) vanhensstumi Goud and Gulden, 2009	Glycymerididae	EA	110	50	Y	N	N	N	N	NC
Empressostrea kostini Huber and Lorenz, 2007	Gryphaeidae	IP	71	500	Y	Y	N	N	H	na
Neopycnodonte zibrowii Gofas, Salas, and Taviani in Wisshak *et al.*, 2009	Gryphaeidae	EA	500	300	Y	Y	N	N	Y	NC
Kelliella abyssicola Allen, 2001	Kelliellidae	EA,WA	4632	ca. 3	Y	Y	N	N	N	na
Kelliella biscayensis Allen, 2001	Kelliellidae	EA	1015	ca. 3	Y	Y	N	N	Y	na
Kelliella concentrica Allen, 2001	Kelliellidae	WA	811	ca. 3	Y	Yd	N	N	N	na
Kelliella elongata Allen, 2001	Kelliellidae	WA	5100	ca. 3	Y	Y	N	N	N	na
Kelliella tenina Allen, 2001	Kelliellidae	EA	1014	ca. 3	Y	Yd	N	N	Y	na
Kellia kussakini Kamenev, 2004b	Kelliidae	NWP	20	4.8	N	N	N	N	N	na
Scacchia rodguesensis Oliver and Holmes, 2004	Lasaeidae	IO	2	3	N	N	N	N	Y	na
Arthritica japonica Lützen and Takahashi, 2003	Leptonidae	NWP	int	2.05	Y	Yd	N	N	Y, H	na
Ctenoides miamiensis Mikkelsen and Bieler, 2003	Limidae	WA	501	13.6	Y	Y	N	N	N	R
Ctenoides obliquus Mikkelsen and Bieler, 2003	Limidae	WA	1335	40	Y	Yd	N	N	N	R
Ctenoides vokesi Mikkelsen and Bieler, 2003	Limidae	WA	732	60	N	N	N	N	N	R
Limatula bisecta Allen, 2004	Limidae	EA	479	2	Y	Y	N	N	Y	na
Limatula celtica Allen, 2004	Limidae	EA	4632	6	Y	Y	N	N	N	na
Limatula demiradiata Allen, 2004	Limidae	EA	330	3.6	Y	Y	N	N	Y	na
Limatula domaneschii de Castro Oliveira and Absalão, 2008	Limidae	WA	1044	1.2	N	N	N	N	Y	na
Limatula margaretae Allen, 2004	Limidae	EA,WA	4632	6	Y	Y	N	N	N	na
Limatula smithi Allen, 2004	Limidae	AN	3385	11.7	Y	Y	N	N	N	na
Limatula thalassae Allen, 2004	Limidae	EA	511	5.3	Y	Y	N	N	N	na
Limea argentineae Allen, 2004	Limidae	WA	993	6.5	Y	Y	N	N	Y	na
Limea lirata Allen, 2004	Limidae	WA	2095	2	Y	Y	N	N	Y	na
Limopsis oliveri Amano and Lutaenko, 2004	Limidae	NWP	218	11.1	N	N	N	N	E	na
Acesta maui Marshall, 2001	Limidae	IP	1172	185	Y	N	N	N	N	R
Acesta oophaga Järnegren, Schander, and Young, 2007	Limidae	WA	800	113	Y	Y	Y	P	Y, H	na
Acesta saginata Marshall, 2001	Limidae	IP	1650	116	Y	N	N	N	N	R
Limopsis marerubra Oliver and Zuschin, 2000	Limopsidae	IO	19	2.5	N	N	N	N	Y	NC
Nipponolimopsis littoralis Sasaki and Haga, 2007	Limopsidae	NWP	int	3	Y	Y	N	N	N	na
Aftolucina discontinua Cosel, 2006	Lucinidae	EA	230	18	Y	N	N	N	H	na
Anodontia aurora Taylor and Glover, 2005	Lucinidae	IP	90	12	N	N	N	N	Y	R
Anodontia blanquita Taylor and Glover, 2005	Lucinidae	EP	46	83	N	N	N	N	N	R

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Anodontia chevalieri Cosel in Taylor and Glover, 2005[10]	Lucinidae	EA	3	42	N	N	N	N	N	R
Anodontia insulosa Taylor and Glover, 2005	Lucinidae	IP	47	30	N	N	N	N	N	R
Anodontia kora Taylor and Glover, 2005	Lucinidae	IO	sh	56	N	N	N	N	N	R
Anodontia senegalensis Cosel in Taylor and Glover, 2005[10]	Lucinidae	EA	230	37	N	N	N	N	N	R
Anodontia trulla Taylor and Glover, 2005	Lucinidae	IP	sh	52	N	N	N	N	Y	R
Anodontia tuticorina Taylor and Glover, 2005	Lucinidae	IO	46	24	N	N	N	N	Y	R
Bathyaustriella thionipta Glover, Taylor, and Rowden, 2004	Lucinidae	IP	500	48.5	Y	Yd	Y	P	Y, H	na
Bretskya scapula Glover and Taylor, 2007	Lucinidae	IP	140	6.9	N	N	N	N	N	NC
Cardiolucina undula Glover and Taylor, 2007	Lucinidae	IP	200	5	N	N	N	N	Y	NC
Divaricella chavani Cosel, 2006	Lucinidae	EA	6	28	N	N	N	N	N	na
Epicodakia nodulosa Glover and Taylor, 2007	Lucinidae	IP	58	15.2	N	N	N	N	N	NC
Ferrocina multiradiata Glover and Taylor, 2007	Lucinidae	IP	417	16.6	N	N	N	N	N	NC
Goniomyrtea avia Glover and Taylor, 2007	Lucinidae	IP	200	10	N	N	N	N	N	NC
Goniomyrtea fidelis Glover and Taylor, 2007	Lucinidae	IP	120	10.4	N	N	N	N	N	NC
Graecina colombiensis Taylor and Glover, 2009	Lucinidae	WA	366	51.6	N	N	N	N	Y	na
Graecina karinae Cosel, 2006	Lucinidae	EA	425	40	N	N	N	N	Y	na
Joellina dosiniformis Cosel, 2006	Lucinidae	EA	425	32	N	N	N	N	Y, H	na
Jorgenia gracile Taylor and Glover, 2009	Lucinidae	WA	600	43.7	N	N	N	N	Y	na
Jorgenia louisiana Taylor and Glover, 2009	Lucinidae	WA	555	60.7	N	N	N	N	H	na
Jorgenia luteophila Taylor and Glover, 2009	Lucinidae	WA	850	27.1	N	N	N	N	Y	na
Lamellolucina jawa Taylor and Glover, 2002	Lucinidae	IP	50	22	N	N	N	N	Y	R
Lamellolucina oliveri Taylor and Glover, 2002	Lucinidae	IO	285	21	N	N	N	N	N	R
Lamellolucina pilbara Taylor and Glover, 2002	Lucinidae	IO	30	22.8	Y	Y	N	N	Y	R
Lamellolucina trisulcata Taylor and Glover, 2002	Lucinidae	IP,IO	20	13.5	N	N	N	N	N	R
Lamylucina exgaini Cosel, 2006	Lucinidae	EA	sh	20	N	N	N	N	N	na
Lepidolucina belepia Glover and Taylor, 2007	Lucinidae	IP	42	12.5	N	N	N	N	Y	NC
Leucospiaera diapliana Glover and Taylor, 2007	Lucinidae	IP	250	5.3	N	N	N	N	N	NC
Liralucina craticula Glover and Taylor, 2007	Lucinidae	IP	8	3.8	N	N	N	N	Y	NC

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Liralucina lifouina Glover and Taylor, 2007	Lucinidae	IP	30	6.9	Y	N	N	N	Y	NC
Liralucina vanbani Glover and Taylor, 2007	Lucinidae	IP	155	3.1	N	N	N	N	Y	NC
Lucinoma anemiophila Holmes, Oliver, and Sellanes, 2005	Lucinidae	EP	780	61	N	N	N	N	Y, H	NC
Lucinoma atalantae Cosel, 2006	Lucinidae	EA	2160	29	Y	N	N	N	Y, H	na
Lucinoma gagei Oliver and Holmes, 2006a	Lucinidae	IO	967	62	Y	Y	N	N	Y	na
Lucinoma kazani Salas and Woodside, 2002	Lucinidae	EA	1709	38.4	Y	Yd	N	N	Y	NC
Lucinoma myriamae Cosel, 2006	Lucinidae	EA	425	53	N	N	N	N	Y, H	na
Meganodontia acetabulum Bouchet and Cosel, 2004	Lucinidae	NWP	256	150	N	N	N	N	Y	na
Myrtina leptolira Glover and Taylor, 2007	Lucinidae	IP	200	14.9	N	N	N	N	N	NC
Myrtina porcata Glover and Taylor, 2007	Lucinidae	IP	174	10.3	Y	N	N	N	Y	NC
Neophysema aphanes Taylor and Glover, 2005	Lucinidae	EP	120	16.6	N	N	N	N	Y	na
Notomyrtea vincentia Glover and Taylor, 2007	Lucinidae	IP	200	7.7	N	N	N	N	Y	NC
Parvidontia laevis Glover and Taylor, 2007	Lucinidae	IP	59	10.9	N	N	N	N	Y	NC
Pilucina australis Glover and Taylor, 2001	Lucinidae	IO,IP	int	4.8	N	N	N	N	N	R
Pillucina copiosa Glover and Taylor, 2007	Lucinidae	IP	120	4.2	Y	N	N	N	Y	NC
Pillucina denticula Glover and Taylor, 2001	Lucinidae	IO	50	3.7	N	N	N	N	Y	R
Pillucina mauritiana Glover and Taylor, 2001	Lucinidae	IO	na	11.6	N	N	N	N	Y	R
Pillucina pacifica Glover and Taylor, 2001	Lucinidae	IP	30	8	N	N	N	N	N	R
Poumea coselia Glover and Taylor, 2007	Lucinidae	IP	70	8.5	N	N	N	N	Y	NC
Solelucina koumacia Glover and Taylor, 2007	Lucinidae	IP	120	1.8	Y	Y	N	N	Y	NC
Tinalucina aequatorialis Cosel, 2006	Lucinidae	EA	98	6.8	N	N	N	N	Y	na
Nucinella boucheti La Perna, 2005	Manzanellidae	IP	1610	25	Y	N	N	N	Y	NC
Mysella gregaria Rotvit, Lützen, Jespersen, and Fox, 2007	Montacutidae	WA	int	6	Y	Yd	N	N	H	na
Mysella narchii Passos and Domaneschi, 2006	Montacutidae	AN	25	3.1	Y	Yd	N	N	Y	NC
Hunkydora rakiura Marshall, 2002	Myochamidae	IP	360	5.2	Y	N	N	N	N	na
Myadoropsis wairua Marshall, 2002	Myochamidae	IP	550	5.1	N	N	N	N	N	na
Bathymodiolus anteumbonatus Cosel, 2008a	Mytilidae	IP	1629	60	Y	Yd	Y	N	Y, H	NC
Bathymodiolus edisonensis Cosel, 2008a	Mytilidae	IP	1629	163	Y	Yd	N	N	Y, H	NC
Bathymodiolus hirtus T. Okutani, Fujikura, and Sasaki, 2004	Mytilidae	NWP	644	81.2	Y	Yd	N	N	Y, H	NC

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Bathymodiolus manusensis Hashimoto and Furuta, 2007	Mytilidae	IP	1900	100.8	Y	Yd	Y	N	Y, H	NC
Bathymodiolus marisindicus Hashimoto, 2001	Mytilidae	IO	2450	86	Y	Yd	N	N	Y, H	NC
Bathymodiolus mauritanicus Cosel, 2002	Mytilidae	EA	1200	110	Y	N	N	N	Y	R
Bathymodiolus securiformis T. Okutani, Fujikura, and Sasaki, 2004	Mytilidae	NWP	642	135.1	Y	Yd	N	N	Y, H	NC
Bathymodiolus taiwanensis Cosel, 2008b	Mytilidae	NWP	355	56	Y	Yd	N	(P)	Y, H	NC
Bathymodiolus tangaroa Cosel and Marshall, 2003	Mytilidae	IP	1205	199.6	Y	Yd	N	N	N	na
Bathymodiolus tangaroa turkayi Cosel, 2008a	Mytilidae	IP	1629	120	Y	Yd	Y	N	Y, H	NC
Benthomodiolus geikotsucola T. Okutani and Miyazaki, 2007	Mytilidae	NWP	4037	43.5	Y	Yd	N	N	Y, H	na
Dacrydium leucoguttatum van der Linden and Moolenbeek, 2004	Mytilidae	WA	25	3	N	N	N	N	N	CK
Gigantidas gladius Cosel and Marshall, 2003	Mytilidae	IP	755	316	Y	Y	N	N	H	na
Gigantidas horikoshii Hashimoto and Yamane, 2005	Mytilidae	NWP	762	195.6	Y	Yd	N	N	Y, H	NC
Lithophaga punctata Kleemann and Hoeksema, 2002	Mytilidae	IP	sh	13.5	Y	N	N	N	N	NC
Musculus nipponicus Kuroda in T. Okutani, 2005	Mytilidae	NWP	53	8.1	N	N	N	N	N	na
Musculus panhai Moolenbeek, 2009	Mytilidae	IP	6	13.4	Y	N	N	N	N	NC
Rhomboidiella rodriguesensis Oliver and Holmes, 2004	Mytilidae	IO	int	3	Y	N	N	N	Y	na
Neilonella profunda T. Okutani and Fujiwara, 2005	Neilonellidae	NWP	7320	6.7	Y	N	N	N	Y	na
Epilepton elpis Allen, 2007	Neoleptonidae	WA	4833	1.04	Y	Y	N	N	N	na
Neolepton amatoi Zelaya and Ituarte, 2004	Neoleptonidae	EP	27	2.4	N	N	N	N	Y	R
Neolepton arjanbosi van der Linden, 2003	Neoleptonidae	EA	int	1.3	N	N	N	N	Y	CK
Neolepton bonaerense Zelaya and Ituarte, 2004	Neoleptonidae	WA	77	2.3	N	N	N	N	Y	R
Neolepton faberi van der Linden, 2003	Neoleptonidae	WA	45	1.9	N	N	N	N	N	CK
Neolepton georgianum Zelaya and Ituarte, 2003	Neoleptonidae	AN	94	3.4	Y	Y	N	N	Y	NC
Neolepton holmergi Zelaya and Ituarte, 2003	Neoleptonidae	AN	94	2.8	Y	Y	N	N	Y	NC
Neolepton moolenbeeki van der Linden, 2003	Neoleptonidae	EA,WA	125	1.8	N	N	N	N	N	CK
Neolepton peetersae van der Linden, 2003	Neoleptonidae	WA	25	2	N	N	N	N	N	CK
Neolepton profundorum Allen, 2000	Neoleptonidae	WA	2323	2.5	Y	Y	N	N	Y	na
Neolepton victor van der Linden, 2003	Neoleptonidae	EA	int	2.7	N	N	N	N	E	CK

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Neolepton yangan Zelaya and Ituarte, 2004	Neoleptonidae	EP	27	2.6	N	N	N	N	Y	R
Crassostrea hongkongensis Lam and Morton, 2003	Ostreidae	NWP	sh	160	Y	N	Y	P	N	C
Pandora gorii Rolán and Hernandez, 2007	Pandoridae	EP	30	5.1	N	N	N	N	Y	na
Panacca chilensis Coan, 2000	Paralimyidae	EP	180	21	N	N	N	N	Y	na
Aequipecten comutatus peripheralis Dijkstra and Kilburn, 2001	Pectinidae	IO	500	20	N	N	N	N	N	NC
Anguipecten pacificus Dijkstra, 2002a	Pectinidae	IP	455	60	N	N	N	N	N	NC
Anguipecten simoneae Morrison and Whisson, 2009	Pectinidae	IO	440	3	Y	N	N	N	Y	C
Cyclochlamys austrina Dijkstra and Marshall, 2008	Pectinidae	IP	415	2.45	N	N	N	N	Y	R
Cyclochlamys bacata Dijkstra and Marshall, 2008	Pectinidae	IP	437	1.9	N	N	N	N	Y	R
Cyclochlamys delli Dijkstra and Marshall, 2008	Pectinidae	IP	1006	5	Y	N	N	N	Y	R
Cyclochlamys irregularis Dijkstra and Marshall, 2008	Pectinidae	IP	622	3	N	N	N	N	Y	R
Cyclochlamys munida Dijkstra and Marshall, 2008	Pectinidae	IP	415	2.15	N	N	N	N	Y	R
Cyclochlamys pileolus Dijkstra and Marshall, 2008	Pectinidae	IP	538	1.5	Y	N	N	N	Y	R
Cyclochlamys plectofilum Oliver and Holmes, 2004	Pectinidae	IO	17	2	N	N	N	N	Y	na
Cyclochlamys wanganellica Dijkstra and Marshall, 2008	Pectinidae	IP	133	2.55	Y	N	N	N	Y	R
Mirapecten tuberosus Dijkstra and Kilburn, 2001	Pectinidae	IO	70	41.6	Y	N	N	N	N	NC
Palliolum minutulum Dijkstra and Southgate, 2000	Pectinidae	IP	14	9.2	Y	N	N	N	N	NC
Veprichlamys africana Dijkstra and Kilburn, 2001	Pectinidae	IO	500	36	N	N	N	N	N	NC
Veprichlamys deynzerorum Dijkstra, 2004	Pectinidae	IP	40	43	Y	N	N	N	Y	na
Adacnarca polarsterni Egorova, 2003	Philobryidae	AC	405	7.7	Y	Yd	N	N	N	na
Cosa tholiatus Oliver and Holmes, 2004	Philobryidae	IO	18	1.60	N	N	N	N	Y	na
Cratis thylicus Oliver and Holmes, 2004	Philobryidae	IO	17	1.6	N	N	N	N	Y	na
Xylophaga bayeri R. Turner, 2002	Pholadidae	WA	365	8	Y	Yd	N	N	N	R
Xylophaga corona Voight, 2007	Pholadidae	EP	2701	8.5	Y	Yd	N	N	Y, H	NC
Xylophaga depalmai R. Turner, 2002	Pholadidae	WA	152	9.8	Y	Y	N	N	N	R
Xylophaga gerda R. Turner, 2002	Pholadidae	WA	2072	3	Y	Yd	N	N	N	R
Xylophaga heterosiphon Voight, 2007[11]	Pholadidae	EP	2750	1.3	Y	Yd	N	N	N	na
Xylophaga microchira Voight, 2007	Pholadidae	EP	2658	3.2	Y	Yd	N	N	N	na
Xylophaga muraokai R. Turner, 2002[11]	Pholadidae	EP	1615	14	Y	Yd	N	N	Y	R
Xylophaga oregona Voight, 2007	Pholadidae	EP	2211	8.2	Y	Yd	N	N	N	na
Xylophaga pacifica Voight, 2007	Pholadidae	EP	2700	6.3	Y	Yd	N	N	N	na

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Xylophaga profunda R. Turner, 2002	Pholadidae	WA	1722	10.9	Y	N	N	N	Y	R
Xylophaga siebenalleri Voight, 2007	Pholadidae	EP	2750	6	Y	Yd	N	N	N	na
Xylophaga tipperi R. Turner, 2002	Pholadidae	WA	152	9	Y	Yd	N	N	Y	R
Xylophaga whoi R. Turner, 2002	Pholadidae	WA	914	14.9	Y	Y	N	N	N	R
Xylophaga zierenbergi Voight, 2007	Pholadidae	EP	3232	15.7	Y	Yd	N	N	Y	na
Xylopholas crooki Voight, 2007	Pholadidae	EP	2656	1.5	Y	Yd	N	N	Y	na
Xylopholas scrippsorum Voight, 2007	Pholadidae	EP	2400	4.4	Y	Yd	N	N	N	na
Cetomya bacata Krylova, 2001	Poromyidae	IP	694	12.8	Y	Y	N	N	N	NC
Cetomya celsa Krylova, 2001	Poromyidae	IP	1980	10.3	Y	Yd	N	N	N	NC
Cetomya nataliae Krylova, 2001	Poromyidae	IP	800	9.4	Y	Y	N	N	N	NC
Cetomya poutiersi Krylova, 2001	Poromyidae	IP	490	97	Y	Yd	N	N	N	NC
Cetomya voskresenskii Krylova, 2001	Poromyidae	IP	980	13.2	Y	Yd	N	N	N	NC
Dilemma japonicum Sasaki and Leal, 2008	Poromyidae	NWP	273	9.3	N	N	N	N	Y	na
Ledella marisnostri La Perna, 2004	Pristiglomidae	EA	976	2.27	N	N	N	N	E	CK
Catillopecten tasmani Dijkstra and Marshall, 2008	Propeamussiidae	IP	1373	10	Y	N	N	N	Y	R
Cyclopecten capverdensis Dijkstra and Goud, 2002	Propeamussiidae	EA	610	7	N	N	N	N	Y	NC
Cyclopecten cincinnatus Dijkstra and Gofas, 2004	Propeamussiidae	EA	845	5	Y	N	N	N	H	NC
Cyclopecten fluctuosus Dijkstra and Marshall, 2008	Propeamussiidae	IP	1799	6.55	Y	N	N	N	Y	R
Cyclopecten textus Dijkstra and Marshall, 2008	Propeamussiidae	IP	2830	12.5	N	N	N	N	Y	R
Cyclopecten vimineus Dijkstra and Gofas, 2004	Propeamussiidae	EA	1500	15	N	N	N	N	H	NC
Parvamussium aldeynzeri Dijkstra, 2004	Propeamussiidae	IP	bat	10	N	N	N	N	Y	na
Parvimussium cancellorum Dijkstra and Marshall, 2008	Propeamussiidae	IP	774	7	N	N	N	N	Y	R
Parvamussium intuslaevis Dijkstra and Gofas, 2004	Propeamussiidae	EA	325	5	N	N	N	N	H	NC
Parvamussium musorstomi Dijkstra, 2001	Propeamussiidae	IP	500	8	N	N	N	N	Y	NC
Parvamussium richer Dijkstra, 2001	Propeamussiidae	IP	591	13	Y	N	N	N	E	NC
Parvamussium toyoshiomaruae T. Okutani, 2005	Propeamussiidae	NWP	311	10.5	N	N	N	N	N	na
Similipecten redferni Dijkstra, 2002b	Propeamussiidae	WA	60	4.5	N	N	N	N	Y	NC
Sinepecten segonzaci Schein, 2006[12]	Propeamussiidae	IP	1674	28	Y	N	N	N	Y, H	na
Gari juliae Willan and Huber, 2007	Psammobiidae	IP	60	36	N	N	N	N	N	na
Gari sharabatiae Rusmore-Villaume, 2005	Psammobiidae	IO	na	25.5	N	N	N	N	E	CK
Abra aegyptiaca Oliver and Zuschin, 2000	Semelidae	IO	40	4	N	N	N	N	N	na
Abrina scarlatoi Kamenev, 2004a	Semelidae	NWP	120	11.2	N	N	N	N	N	na
Solemya (Petrasma) miuraensis Kanie and Kuramochi, 2002	Solemyidae	NWP	20	53.3	N	N	N	N	Y	na
Solemya notialis Simone, 2009a	Solemyidae	WA	33.5	12.9	Y	Yd	N	N	Y	na
Solemya tagiri T. Okutani, Hashimoto, and Miura, 2004	Solemyidae	NWP	116	23.8	Y	Y	N	N	Y, H	NC
Solen darwinensis Cosel, 2002b	Solenidae	IP	sh	83.6	N	N	N	N	E	R

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Solen kikuchii Cosel, 2002b	Solenidae	NWP	49	25.9	Y	Y	N	N	N	R
Solen pseudolinearis Cosel, 2002b	Solenidae	NWP	sh	64.1	N	N	N	N	Y	R
Solen sarawakensis Cosel, 2002b	Solenidae	IP	sh	134	Y	Y	N	N	N	R
Solen soleneae Cosel, 2002b	Solenidae	NWP,IO	sh	39.5	N	N	N	N	N	R
Solen thachi Cosel, 2002b	Solenidae	IP	sh	75.7	N	N	N	N	N	R
Solen thailandicus Cosel, 2002b	Solenidae	IP	sh	48.9	Y	Y	N	N	Y	R
Grippina acherontis Marshall, 2002	Spheniopsidae	IP	40	2.45	Y	N	N	N	Y	na
Grippina globosa Marshall, 2002	Spheniopsidae	IP	550	1.43	Y	N	N	N	Y	na
Grippina pumila Marshall, 2002	Spheniopsidae	IP	183	2.8	Y	N	N	N	N	na
Grippina punctata Marshall, 2002	Spheniopsidae	IP	805	2.15	Y	N	N	N	N	na
Grippina rex Marshall, 2002	Spheniopsidae	IP	805	5.1	Y	N	N	N	E	na
Grippina spirata Marshall, 2002	Spheniopsidae	IP	200	2.05	Y	N	N	N	N	na
Spondylus cevikeri Lamprell, Stanisic, and Clarkson, 2001b	Spondylidae	EA	10	80.7	N	N	N	N	N	R
Spondylus deforgesi Lamprell and Healy, 2001	Spondylidae	IP	100	34.8	N	N	N	N	Y	NC
Spondylus exignus Lamprell and Healy, 2001	Spondylidae	IP	140	6.4	N	N	N	N	Y	NC
Spondylus heidkeae Lamprell and Healy, 2001	Spondylidae	IO,IP	110	45	N	N	N	N	N	NC
Spondylus maestratii Lamprell and Healy, 2001	Spondylidae	IP	60	45.5	N	N	N	N	N	NC
Spondylus mireilleae Lamprell and Healy, 2001	Spondylidae	IP	460	72.3	N	N	N	N	N	NC
Spondylus orstomi Lamprell and Healy, 2001	Spondylidae	IP	318	48.5	N	N	N	N	Y	NC
Spondylus proueri Lamprell and Healy, 2001[12]	Spondylidae	IP	900	30.9	Y	N	N	N	N	NC
Spondylus rippingalei Lamprell and Healy, 2001	Spondylidae	IO,IP	49	4	N	N	N	N	N	NC
Spondylus swinneni Lamprell, Stanisic, and Clarkson, 2001a	Spondylidae	IP	50	125	N	N	N	N	N	na
Macoma biota Arrua and Domaneschi, 2005	Tellinidae	WA	int	53.4	Y	Yd	N	N	Y	NC
Semelangulus mesodesmoides Oliver and Zuschin, 2000	Tellinidae	IO,IP	10	8	N	N	N	N	N	NC
Asthenothaerus maxwelli Marshall, 2002	Thraciidae	IP	293	46	N	N	N	N	N	na
Parvithracia ampla Marshall, 2002	Thraciidae	IP	1019	6.5	N	N	N	N	N	na
Parvithracia fragilissima Marshall, 2002	Thraciidae	IP	1386	7.9	Y	N	N	N	N	na
Parvithracia lukini Kamenev, 2002	Thraciidae	NWP	418	11	Y	Y	N	D	N	na
Parvithracia melchior Marshall, 2002	Thraciidae	IP	805	11.5	N	N	N	N	N	na
Parvithracia sirenkoi Kamenev, 2002	Thraciidae	NWP	920	8.9	Y	Y	N	D	N	na
Thracia arienatoma Oliver and Holmes, 2004	Thraciidae	IO	na	3	Y	N	N	N	Y	na
Trigonothracia mimica Marshall, 2002	Thraciidae	IP	415	3.95	Y	N	N	N	N	na
Adontorhina keegani Barry and McCormack, 2007	Thyasiridae	EA	789	0.98	Y	Yd	N	N	Y	NC
Adontorhina similis Barry and McCormack, 2007	Thyasiridae	EA	382	1.25	Y	Yd	N	N	N	NC

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Axinus cascadiensis Oliver and Holmes, 2007	Thyasiridae	EP	2592	23.6	Y	Yd	N	N	Y, H	NC
Conchocele novaeguinensis K. Okutani, 2002	Thyasiridae	IP	470	73.9	N	N	N	N	Y	NC
"Leptaxinus" indusarium Oliver and Levin, 2006	Thyasiridae	IO	1000	5	Y	Y	N	N	Y	NC
Spinaxinus sentosus Oliver and Holmes, 2006b	Thyasiridae	EA	1160	16.5	Y	Y	N	N	Y	NC
Thyasira methanophila Oliver and Sellanes, 2005	Thyasiridae	EP	780	29.7	Y	Y	N	N	H	na
Thyasira scotiana Zelaya, 2009	Thyasiridae	AN	na	10	Y	Y	N	N	Y	na
Thyasira southwardae Oliver and Holmes, 2006b	Thyasiridae	AC	3040	16.7	Y	Y	N	N	Y, H	NC
Thyasira volcolutre Rodrigues and Oliver in Rodrigues, Oliver, and Cunha, 2008	Thyasiridae	EA	2200	17.2	Y	Yd	Y	P	H	NC
Diplodonta bogii Van Aartsen, 2004[13]	Ungulinidae	EA	na	10	N	N	N	N	N	na
Diplodonta moolenbeeki Van Aartsen and Goud, 2006	Ungulinidae	EA	na	25	N	N	N	N	N	R
Bonartemis amamiensis T. Okutani, 2005	Veneridae	NWP	126	20.5	N	N	N	N	Y	na
Lioconcha berthaulti Lamprell and Healy, 2002	Veneridae	IP	53	31.7	N	N	N	N	Y	NC, R
Lioconcha macaulayi Lamprell and Healy, 2002	Veneridae	IP	4	47.4	N	N	N	N	Y	NC, R
Lioconcha pseudofastigiata Lamprell and Healy, 2002	Veneridae	IP	sh	43.7	N	N	N	N	Y	NC, R
Lioconcha schioettei Lamprell and Healy, 2002	Veneridae	IP	52	42.1	N	N	N	N	N	NC, R
Microcirce consternana Oliver and Zuschin, 2001	Veneridae	IO	30.5	3.3	Y	Y	N	N	N	na
Spinosipella agnes Simone and Cunha, 2008	Verticordiidae	WA	900	22	N	N	N	N	N	R
Spinosipella tinga Simone and Cunha, 2008	Verticordiidae	WA	253	16.9	N	N	N	N	N	R
Verticordia ouricuri de Castro Oliveira and Absalão, 2009	Verticordiidae	WA	1950	4.5	N	N	N	N	Y	R
Callogonia cyrili Cosel and Salas, 2001	Vesicomyidae	EA	1805	26	N	N	N	P	Y	NC
Callogonia mauritanica Cosel and Salas, 2001	Vesicomyidae	EA	1200	7.6	N	N	N	P	Y	NC
Calyptogena (Archivesica) edisonensis T. Okutani, Kojima, and Kim, 2004	Vesicomyidae	IP	1483	99.8	Y	N	N	N	Y, H	NC
Calyptogena (Archivesica) magnocultellus K. Okutani, Kojima, and Iwasaki, 2002	Vesicomyidae	NWP	2535	198	N	N	N	N	Y	NC
Calyptogena (Archivesica) tsubasa T. Okutani, Fujikura, and Kojima, 2000	Vesicomyidae	NWP	3800	212.1	N	N	Y	N	Y	NC

Appendix. (Continued)

Species	Family	Zone[1]	Dep[2]	Size[3]	Live[4]	Anat[5]	Mol[6]	Anl[7]	Res[8]	Source[9]
Calyptogena (Ectenagena) fossajaponica T. Okutani, Fujikura, and Kojima, 2000	Vesicomyidae	NWP	6809	31.9	N	N	N	N	Y	NC
Calyptogena gallardoi Sellanes and Krylova, 2005	Vesicomyidae	EP	760	45	N	N	N	N	Y, H	NC
Calyptogena garuda T. Okutani and Soh, 2005	Vesicomyidae	IP	2064	237	N	N	N	N	Y	na
Isorropodon bigoti Cosel and Salas, 2001	Vesicomyidae	EA	1200	21	Y	Y	N	P	N	NC
Isorropodon curtum Cosel and Salas, 2001	Vesicomyidae	EA	1200	11.3	N	N	N	P	Y	NC
Vesicomya crenulomarginata Okutani, Kojima, and Iwasaki, 2002	Vesicomyidae	NWP	2517	47	N	N	N	N	Y	NC
Vesicomya kaikoae K. Okutani, Fujikura, and Kojima, 2000	Vesicomyidae	NWP	3760	42.3	N	N	N	N	Y	NC
Vesicomya kuroshimana K. Okutani, Fujikura, and Kojima, 2000	Vesicomyidae	NWP	812	58.9	N	N	N	N	Y	NC
Waisiuconcha haeckeli Cosel and Salas, 2001	Vesicomyidae	EA	1200	4	Y	N	N	P	Y	NC
Yoldiella dautzenbergi La Perna, 2008	Yoldiidae	EA	1360	4.08	N	N	N	N	Y	na
Yoldiella kaikonis T. Okutani and Fujiwara, 2005	Yoldiidae	NWP	7333	6.6	Y	Yd	N	N	Y	na
Yoldiella ovulum La Perna, 2004	Yoldiidae	EA	1500	1.9	N	N	N	N	E	CK
Yoldiella thaerella Killeen and J. Turner, 2009	Yoldiidae	AC	2900	3.6	Y	Y	N	N	N	NC
Yoldiella wareni La Perna, 2004	Yoldiidae	EA	2000	1.51	N	N	N	N	E	CK

[1] Zones: AC, Arctic; AN, Antarctic; EA, eastern Atlantic; EP, eastern Pacific; IO, Indian Ocean; IP, Indo-West Pacific; NWP, northwestern Pacific; WA, western Atlantic.

[2] Dep: maximum recorded depth (m) for live- and dead-collected specimens; bat, bathyal; int, intertidal; sh, shallow.

[3] Size: maximum recorded dimension.

[4] Live: original material includes live-collected specimens.

[5] Anat: original description includes anatomical characters; Yd, anatomical characters are diagnostic for the new species.

[6] Mol: original description includes molecular characters.

[7] Anl: original description publication includes an analysis; D, discriminant; P, phylogenetic; (P), new species related to a previous phylogenetic analysis.

[8] Res: species is restricted to a particular geographical distribution (Y) or habitat (H); E, stated as "endemic" in the original description.

[9] Source: source of the new material; C, commercial harvesting or aquaculture; CK, in context of revision of a regional checklist; NC, new collections; R, in context of a taxonomic revision (in some cases regional).

[10] Redescribed by Cosel (2006).

[11] Additional data from Voight (2009).

[12] Additional data from Dijkstra and Marshall (2008).

[13] Additional data from van Aartsen and Goud (2006).

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What, if anything, can we learn from the fossil record about speciation in marine gastropods? Biological and geological considerations*

Warren D. Allmon[1,2] and Ursula E. Smith[2]

[1] Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, New York 14850, U.S.A.

[2] Department of Earth and Atmospheric Sciences, Cornell University, Ithaca, New York 14853, U.S.A.

Correspondence, W. D. Allmon: wda1@cornell.edu

Abstract: Using fossils to study speciation requires careful analysis of the potential and limits of both biological and geological data. The most important biological data for a particular taxon includes to what degree species can be distinguished based only on hard-part morphology, what processes lead to speciation in that group today, and at what rates speciation occurs. Among the most important geological considerations is whether the incompleteness of the fossil record makes it possible to specify to within a reasonable degree of confidence when and where a putative speciation event took place. In benthic marine macroinvertebrates, the latter analysis is complicated by the "Common Cause" phenomenon: sea level change is both a major potential cause of gaps in the record and an important potential cause of evolutionary change. We consider the potential and limitations of fossil data for providing unique insight into the patterns and processes of speciation in marine shelled gastropods. A review of biological data shows considerable variation in mode of speciation and correlation between shell morphology and genetic divergence (cryptic species). Through examination of the fossil records of turritelline gastropods (Cerithioidea, Turritellidae) from Cenozoic deposits of the U.S. Gulf and Atlantic Coastal Plains and New Zealand, we propose simple tests for judging whether a particular fossil record is adequate for drawing conclusions about pattern and process of speciation. We conclude that the fossil record of marine gastropods *can* provide valuable information for studying species and speciation, but only if great care is used in evaluation of the data.

Key words: unconformities, completeness, cryptic species, Turritellidae

The role of the fossil record in investigating the origin of species is complex (Benton and Pearson 2001, Miller 2006). On one hand, species are at the center of paleontological studies of evolutionary tempo and mode (*e.g.*, Jackson and Cheetham 1999, Gould 2002), and have featured prominently in many paleontological studies of pattern and process in origination and extinction (*e.g.*, Jablonski 1986a, 1986b, Sepkoski 1998, Benton 2009). On the other, the fossil record is famously incomplete, and the degree to which fossil species are the same as those recognized in studies of modern organisms remains controversial, even among paleontologists (*e.g.*, Jablonski *et al.* 1986, Smith 1994, Levinton 2001, Pearson and Harcourt-Brown 2001, Coyne and Orr 2004).

Given this complexity, it is surprising that many paleontological studies have claimed to be addressing patterns and causes of "speciation" (albeit sometimes referred to more agnostically as "origination" or "diversification") without explicitly discussing whether they are really studying the same phenomena that neontologists call by the same name. Numerous paleontological studies, covering the entire Phanerozoic and virtually all preserved animal phyla, have hypothesized extrinsic or intrinsic causes for the origin of species (*e.g.*, Eldredge 1971, Hallam 1982, Ager 1983, Valentine and Jablonski 1983, Bayer and McGhee 1985, Cronin 1985, Gingerich 1985, Reif 1985, Vrba 1985, McRoberts and Aberhan 1997, Sandoval *et al.* 2001, McCune 2004, Alroy 2009). Presumably, these authors used the terms "species" and "speciation" assuming some connection to their use in studies of modern taxa. Yet there have been very few detailed examinations of exactly what "species" or "speciation" actually mean in such paleontological investigations (*e.g.*, Jackson and Cheetham 1999).

Gastropods are an especially important group in which to consider the role of the fossil record in elucidating evolutionary pattern and process at the species level. Their shells are commonly well-preserved and abundant in fossil assemblages, and they are among the most diverse clades of the past 100 million years (Erwin and Signor 1991, Morris and Taylor 2000). Fossil gastropods have been the subject of numerous influential studies of origination and extinction at the species

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level (*e.g.*, Gould 1969, Kauffman 1977, Hansen 1978, 1980, Williamson 1981, Jablonski 1986a, 1986b, 1987, 1997, Hansen *et al.* 1987, Erwin 1989, Roy 1994, Jablonski and Roy 2002, Glaubrecht 2011). The form of their shells, furthermore, has encouraged species-level paleontological studies of the relationship between form, development, and evolution (*e.g.*, Gould 1969, 1989, Geary 1988, Allmon 1994).

In this paper, we try to assess the feasibility of studying speciation in the fossil record by asking two questions: (1) what are the challenges in studying species and speciation in fossil marine gastropods, and how can they be overcome? (2) What can we learn about species and speciation in gastropods from such studies that we would not be able to learn (at all, or as easily) from studying modern forms? To address these two questions, we first undertake a review of the relevant *biological* issues—species and speciation in living marine gastropods (Krug 2011)—to see what generalizations might be applied (using uniformitarian reasoning) to fossils. We then undertake a mostly *geological* analysis of the options for confronting perhaps the most frequent obstacle in paleontological studies of speciation—incomplete preservation of stratigraphic and geographic ranges due to gaps in the fossil record—and we apply a set of simple tests for completeness to the fossil record of turritelline gastropods. We conclude with some tentative conclusions and a set of recommendations for how to study speciation in fossil marine gastropods in the future.

We devote most of our attention here to marine shelled gastropods (mostly vetigastropods and caenogastropods) because these are the most common gastropods in the fossil record. Our conclusions may or may not apply equally to the fossil records of other taxa, such as those on land or in freshwater (*e.g.*, Gould 1969, Reif 1985, Willmann 1985, Nützel and Bandel 1993, Glaubrecht 2011).

PREVIOUS STUDIES

What might be called "pre-modern" studies of species-level evolution in fossil marine gastropods (*e.g.*, Grabau 1904, Clark 1945, Fisher *et al.* 1964, Rodda and Fisher 1964) focused mainly on species description, stratigraphic distribution, and hypothesized anagenetic ancestor-descendant sequences based on stratophenetic phylogenies. Cladogenesis was not emphasized if it was mentioned at all.

More recent studies of speciation using the fossil record of marine gastropods can be placed in four categories. First are studies of single lineages or small subclades (*e.g.*, Hansen 1978, 1980, Schindel 1982, Allmon 1990, Geary 1990a, 1990b, 1995, Nehm and Geary 1994, McKinney and Allmon 1995, Gili and Martinell 2000, Kaim 2002). These studies frequently report correlations between environmental change and cladogenesis, but also document the occurrence of anagenesis as an apparent response to changes in environmental conditions. Although they may be satisfyingly specific, as noted by Wagner and Erwin (1995) such studies do not provide tests of the generality or relative frequency of any particular mode of speciation.

A second type of study surveys species across fossil assemblages, faunas, or clades, and attempts to correlate some kind of intrinsic or extrinsic factor with the appearance of new species (*e.g.*, Kohn 1990, Allmon 2001a, Jablonski and Roy 2002). A growing third group consists of similar studies that attempt to link molecular phylogenies, and the timing of cladogenetic events in these phylogenies as determined by molecular clocks, to major environmental perturbations, such as regional tectonism (*e.g.*, Williams and Duda 2008). Both fossil assemblage and molecular phylogenetic studies report positive correlations between environmental change and origination of new species. A fourth approach examines broader phylogenetic patterns, such as the distribution of plesiomorphies and the frequency of polytomies, as a source of insight into modes of speciation (*e.g.*, Wagner 1995, Wagner and Erwin 1995).

In sum, these previous studies of species and speciation in fossil gastropods are consistent with (but do not all require) speciation by allopatry. Most have suggested that cladogenesis is frequently the result of changes in the extrinsic environment, but also that some lineages respond to such changes by anagenetic transformation, in addition to or instead of cladogenesis. The relative frequencies of these various tempos and modes, however, remain unclear, as does the relative role of different environmental factors (sea level, temperature, etc.) in contributing to these different evolutionary patterns.

BIOLOGICAL ISSUES

Species recognition

The problem of taxonomic recognition and delimitation in shelled gastropods has long been recognized (*e.g.*, Gray 1835, Schander and Sundberg 2001, Wagner 2001). As has frequently been noted (but seldom documented), the vast majority of living shelled gastropod species descriptions have been (and continue to be) based exclusively on shell characters. This situation has led to the observation by paleontologists that fossil species (not just of gastropods) are effectively no different from most modern species (*e.g.*, Allmon 1990, Gould 2002: 785). When non-shell morphological characters, such as radula and soft-part anatomy, have been examined, however, such "conchological morphospecies" among caenogastropods are sometimes validated (*e.g.*, Michaux 1987, 1989, Kantor *et al.* 2008) and sometimes not (*e.g.*, Houbrick 1980, 1981). As molecular sequence data have become more

abundant, the topic of "species delimitation" has become more widely discussed, both in animals in general (Sites and Marshall 2003, 2004, de Queiroz 2007, Knowles and Carstens 2007, Shaffer and Thomson 2007, Wiens 2007, Hausdorf and Hennig 2010, Ross *et al.* 2010, Weisrock *et al.* 2010) and in marine gastropods in particular (*e.g.*, Reid *et al.* 2006, Bichain *et al.* 2007, Haase *et al.* 2007, Puillandre *et al.* 2009, Castelin *et al.* 2010).

Determining to what degree shell morphology reflects genetic divergence is further complicated by our lack of understanding of the causes for variation in shell characters in gastropods (*e.g.*, Olabarria and Thurston 2004, and references therein). Although a number of studies have demonstrated genetic components of morphological variation (*e.g.*, Janson 1982, Palmer 1984, Boulding and Hay 1993, Johannesson *et al.* 1993, Rolán *et al.* 2004), other studies have demonstrated significant ecophenotypic plasticity (*e.g.*, Wigham 1975, Hollander *et al.* 2005, 2006, Freiheit and Geary 2009, and references therein), and sometimes specifically used these conclusions to express varying degrees of caution about the recognition of fossil species (*e.g.*, Kemp and Bertness 1984, Palmer 1985, Palmer *et al.* 1990, Geiger and Groves 1999, Samadi *et al.* 2000).

As is true for other groups, species-level studies of fossil gastropods seldom explicitly state their operational concept of species. Exceptions (*e.g.*, Jung 1986, Allmon 1990, 1996, Nehm and Geary 1994, Freiheit and Geary 2009) usually compare morphometric variation in shell form of nominal modern species to fossils arrayed in time and space; consistently similar patterns are generally taken as sufficient evidence of practical equivalence.

The extreme case of discordance between such paleontologically recognizable morphospecies and genetically distinct evolutionary lineages is the occurrence of sibling or cryptic species. Although numerous examples were long ago identified in a variety of taxa (Mayr 1942, 1963), the recent proliferation of molecular data has led to a dramatic increase in the recognition of cryptic species across the animal kingdom, including in marine gastropods (*e.g.*, Knowlton 1993, 2000, Sáez and Lozano 2005, Bickford *et al.* 2007, Beheregaray and Caccone 2007, Pfenninger and Schwenk 2007, Castelin *et al.* 2010, Pérez-Ponce de Léon and Nadler 2010, Table 1).

Paleontologists usually acknowledge that they can say little about cryptic species (*e.g.*, Vrba 1980: 68, Gould 2002: 785ff, but see Gili and Martinell 2000, and Herbert and Portell 2004). Like most aspects of paleontology, however, the assumption is that knowledge of the occurrence of cryptic species among living forms can provide a reasonable assessment of the occurrence among extinct forms. There are two aspects to this problem. The first is common to all such applications of uniformitarianism: it is possible that the present is not an entirely reliable key to the past (*e.g.*, Kauffman 1987, Bottjer 1998, Allmon 2007). The second is that even if present and past are similar, we frequently know too little about the present. For example, Mayr (1963: 37) suggested that cryptic species "seem to be more common in some groups... than in others", yet this has proven to be controversial. Conflicting recent analyses of cryptic species across all phyla have argued either that the proportion of cryptic species is highly variable among higher taxa (*e.g.*, Bickford *et al.* 2007, Trontelj and Fišer 2009, Poulin 2010), or that it is approximately equal (Pfenninger and Schwenk 2007). If the former, then it might in principle be possible to assess the relative frequency in different taxa, and then apply the results to their respective fossil representatives. If the latter holds, then (at least for comparisons among taxa) cryptic species could be ignored (although we still might not know what the actual absolute frequency is in a particular taxon).

The occurrence of cryptic species in marine gastropods has not previously been the subject of careful analysis, and the list of published reports in Table 1 provides only sparse insight into what the actual pattern might be. It is possible that the taxonomic distribution of cryptic species in Table 1 is representative of the actual distribution among gastropod higher taxa. It is more likely, however, that the abundance of published reports of cryptic species in, for example, *Littorina* and related forms reflects the relatively intense level of study that these taxa have received from ecologists and systematists, as much or more than the actual frequency of cryptic species within the group. Similarly, the seemingly high number of reports among cones and turrids may be related simply to their very high species diversity.

In summary, data currently available support the conclusion that cryptic species do occur widely among living shelled marine gastropods, and that this should be taken into account when thinking about fossils (*e.g.*, speciation rates based on fossils are minimum estimates). At least some instances of otherwise cryptic species can be recognized in fossils based on differences in the larval shell (Gili and Martinell 2000, Herbert and Portell 2004), and this might be a fruitful area for future work. It remains to be determined, however, whether cryptic species are more common in marine gastropods than in other higher taxa, whether all marine gastropod groups show equal proportions of cryptic species, or what the pattern of occurrence of cryptic species was in marine gastropods in the geological past.

Modes of speciation

New species can arise in a variety of ways (Table 2). Most recent studies of speciation in marine gastropods have concluded that speciation is predominantly allopatric (*e.g.*, McManus 1985, Knight *et al.* 1987, Vermeij 1987, Denis *et al.* 1993, Collins *et al.* 1996, Hellberg 1998, Marko 1998, Pudovskis *et al.* 2001, Collin 2003, Meyer 2003, Williams and

Table 1. Published studies documenting cryptic species in shelled marine gastropods. Genera are listed as in the references cited.

Family	Genus	References
Patellidae	*Patella* Linnaeus, 1758	Evans (1953), Ridgway *et al.* (1998)
Lottiidae	*Lottia* Gray, 1833	Crummett and Eernisse (2007)
	Collisella Dall, 1871	Murphy (1978)
	Notoacmaea Iredale, 1915	Simison and Lindberg (1999), Nakano and Spencer (2007)
Lepeteodrilidae	*Lepetodrillus* McLean, 1988	Johnson *et al.* (2008), Vrijenhoek (2009)
Trochidae	*Oxystele* Philippi, 1847	Heller and Dempster (1991)
	Diloma Philippi, 1845	Spencer *et al.* (2009)
	Austrocochlea Fischer, 1885	Parsons (1996)
Turbinidae	*Astralium* Link, 1807	Meyer *et al.* (2005)
	Alviniconcha Okutani and Ohta, 1988	Denis *et al.* (1993)
Provaniidae		
Calyptraeidae	*Calyptraea* Lamarck, 1799	Gallardo (1977, 1979)
	Bostrycapulus Olsson and Harbison, 1953	Collin (2005)
	Crepidula Lamarck, 1799	Gallardo (1977, 1979), Hoagland (1977, 1984, 1986), Collin (2000, 2001), Chaparro *et al.* (2002), Véliz *et al.* (2003), Schmidt *et al.* (2006)
Littorinidae	*Littorina* Ferussac, 1822	Heller (1975), Sacchi *et al.* (1977), Murray (1979), Wilkins and O'Regan (1980), Bandel and Kadolsky (1982), Mastro *et al.* (1982), Moyse *et al.* (1982), Raffaelli (1982), Sacchi (1984), Ward and Janson (1985), Reid (1986), Janson (1987), Chow (1987), Ward (1990), Warwick *et al.* (1990), Boulding *et al.* (1993), Crossland *et al.* (1993), Rolán-Alvarez *et al.* (1995a, 1995b), Grahame *et al.* (1997), Clarke *et al.* (1999), Tatarenkov and Johannesson (1998), Wilding *et al.* (2000), Hohenlohe (2002)
	Nodilittorina Martens, 1897	
	Bembicium Philippi, 1846	Reid (1988)
	Risellopsis Kesteven, 1902	
Rissoidae	*Alvania* Risso, 1826	Oliverio (1996)
	Rissoa Freminville, 1814	Rehfeldt (1968), Munksgaard (1990), Oliverio (1996)
	Frigidoalvania Warén, 1974	Quattro *et al.* (2001)
Vermetidae	*Dendropoma* Mörch, 1861	Safriel and Hadfield (1988), Calvo *et al.* (2009)
	Petaloconchus Lea, 1843	Weinberger *et al.* (2010)
Buccinidae	*Nassaria* Rafinesque, 1815	Castelin *et al.* (2010)
	Sassia Bellardi, 1873	Castelin *et al.* (2010)
Nassariidae	*Nassarius* Dumeril, 1806	Oliverio and Tringali (1992), Oliverio (1996), Sanjuan *et al.* (1997)
Muricidae	*Chicoreus* Montfort, 1810	Castelin *et al.* (2010)
	Nucella Röding, 1798	Palmer *et al.* (1990), Marko (1998)
	Leptoconchus Rueooell, 1834	Gittenberger and Gittenberger (2006)
Volutidae	*Alicithoe* H. and A. Adams, 1853	Castelin *et al.* (2010)
Olividae	*Amalda* H. and A. Adams, 1853	Michaux (1987)
Conidae	*Conus* Linnaeus, 1758	Chaney (1987), Vallejo (2005), Duda *et al.* (2008, 2009)
Turridae	*Mangelia* Risso, 1826	Oliverio (1996)
	Bela Leach, 1847	Oliverio (1996)
	Raphitoma Bellardi, 1848	Oliverio (1996)
	Haedropleura Monterosato, 1883	Oliverio (1996)
	Gemmuloborsonia Shuto, 1989	Puillandre *et al.* (2010)
	Xenuroturris Iredale, 1929	Kantor *et al.* (2008)
Bullidae	*Bulla* Linnaeus, 1758	Malaquias and Reid (2008)

Reid 2004, Donald *et al.* 2005, Meyer *et al.* 2005, Paulay and Meyer 2006, Reid *et al.* 2006, Malaquias and Reid 2009, Castelin *et al.* 2010). Numerous recent authors have also suggested, however, that non-allopatric modes of speciation (especially ecological speciation) are important in marine gastropods (*e.g.*, Hellberg 1998, Pickles and Grahame 1999, Cruz *et al.* 2004, Hollander *et al.* 2005, Vallejo 2005, Conde-Padín *et al.* 2007, Glaubrecht 2009, Johannesson 2009, Krug

Table 2. Modes of speciation (modified from, *inter alia*, Lynch 1989, Wagner and Erwin 1995, de Queiroz 1998, Coyne and Orr 2004).

	Mode	Geography	"Allopatric mechanism"	Parent:daughter population size
I.	Anagenesis			
II.	Cladogenesis			
A.		Allopatric		
1.			Vicariance	
				Parent ≅ daughter (bifurcation, dumbbell allopatry)
				Parent >> daughter (peripatry)
2.			Dispersal	Parent >> daughter (peripatry)
B.		Sympatric		

2011). It remains to be determined what the actual relative frequency is between allopatric and non-allopatric speciation, or whether it varies among clades.

Within the allopatric mode and its two principal geographic mechanisms, vicariance and dispersal, considerable attention has been devoted to the influence of larval biology on probability or rate of speciation in marine gastropods (*e.g.*, Hansen 1978, 1980, Scheltema 1978, Jablonski 1986a, Jablonski and Lutz 1980, 1983). The most widely held view is that species with larvae that spend more time in the plankton (mostly planktotrophs, but including some non-planktotrophs) have higher dispersal probabilities, wider geographic ranges, and lower rates of isolate formation, genetic divergence, and speciation. Species with larvae that spend less or no time in the plankton, on the other hand, are generally thought to have lower dispersal probability, narrower ranges, and higher rates of isolation, divergence, and speciation (*e.g.*, Valentine and Jablonski 1983, Jablonski 1986a). Planktotrophy is widely viewed to be the primitive condition in most clades of caenogastropods (Jablonski and Lutz 1983). Thus, the evolution of non-planktotrophy in a lineage or clade is often seen as an event that may lead to an increase in speciation (*e.g.*, Hansen 1978, 1980, Cunha *et al.* 2005). For the great majority of caenogastropod clades, however, the proportion of species with planktotrophic versus nonplanktotrophic larvae through time is not known in any detail.

The frequently wide geographic distribution of marine invertebrate species has been called a paradox by some authors, who have wondered how isolation can occur in the presence of so much dispersal and (presumably) gene flow (Palumbi 1992, 1994, Neigel 1997, Williams and Reid 2004, Meyer *et al.* 2005). Several solutions to this paradox have been proposed:

(1) Allopatric speciation in many marine environments (especially shallow coastal areas) may frequently occur at smaller geographic scales than previously recognized (Meyer *et al.* 2005).

(2) High dispersal potential does not necessarily imply a paucity of geographic barriers (*e.g.*, Waters *et al.* 2005), nor does low dispersal potential of larvae necessarily imply low dispersal of adults (Donald *et al.* 2005, Imron *et al.* 2007). At least some widespread species may also be more likely to form more isolates (and therefore give rise to more descendant species) simply because they are larger and inhabit wider geographic areas, and perhaps also a wider array of environments (Scheltema 1978).

(3) Diversification in at least some clades may be greatest at intermediate levels of dispersal (Paulay and Meyer 2002, 2006), except at very local scales, where species complexes (clusters of very closely related species) appear to be most frequent among low-dispersal species (Paulay and Meyer 2006, Castelin *et al.* 2010).

(4) Narrow geographic ranges in daughter species might make them more extinction-prone, but might also permit greater "species packing", enhancing survival and allowing for higher local species diversity (*e.g.*, Jablonski and Roy 2002, Meyer *et al.* 2005).

(5) As mentioned above, we may have underappreciated the occurrence of non-allopatric modes of speciation (*e.g.*, Johannesson 2009, Krug 2011).

Other variations of conventional allopatric speciation have been reported in modern marine gastropods. Coincident geographic ranges of apparently ecologically similar species, for example, do not always produce coincident responses to extrinsic environmental disturbance "suggesting that relatively minor differences in traits such as pelagic larval duration or microhabitat association may profoundly impact phylogeographical structure" (Crandall *et al.* 2008: 611). Sister taxa are not always divided by broad and long-lasting geographic barriers; "transient allopatry" or purely ecological barriers may suffice (Hellberg 1998). It has also been suggested that mode of speciation may differ under different geographic conditions; coastal species, for example, may be more likely to show speciation by peripatric dispersal and may favor the sympatry of sister taxa, whereas broad regions with many oceanic islands (such as the modern Indo-West Pacific) may be sites of more vicariant allopatry and less sister species overlap (Valentine and Jablonski 1983, Hellberg 1998).

Thus, living marine gastropods appear to show a variety of modes of speciation. There is clearly ample evidence for speciation by allopatry (via both vicariance and dispersal), but the linkage between larval dispersal and probability of speciation is neither simple nor uniform across taxa. There is, furthermore, strong (and perhaps growing) evidence for the occurrence of sympatric, or ecological, modes of speciation, apparently driven largely by relatively small shifts in habitat preference within otherwise continuous populations. It unfortunately remains unclear, however, whether there are broad intrinsic or extrinsic factors that might bias species formation toward one mode more than another.

Rates of speciation

Discussions about "rates of speciation" focus on two different phenomena, which are not necessarily closely related. The first is the time interval between speciation events within a lineage ("species per million years" or the "speciation interval") (Raup 1978, Coyne and Orr 2004). The second is the time it takes for a daughter population to diverge sufficiently from the parent population to achieve reproductive isolation (the "transition time", "time for speciation", "waiting time to speciation", or "duration of speciation") (Gavrilets 2000, Coyne and Orr 2004, McCune 2004, Curnoe *et al.* 2006). Speciation intervals are generally longer than transition times (and can be *much* longer) because lineages do not begin to branch immediately after they arise. Speciation intervals are estimated in a variety of ways, usually based on the fossil record. Transition times are generally more difficult to estimate, because this requires knowledge of when speciation begins, and so most estimates of "speciation rates" in the literature are actually "speciation intervals".

In rare instances such as lakes that preserve extremely high-resolution fossil records, transition times can be estimated with high precision (*e.g.*, McCune 2004). Estimates of "divergence times" between geminate species that are commonly used to calibrate molecular clocks (*e.g.*, Marko 2002)—equivalent to estimates of "speciation duration" in phylogeography (Avise 2000)—are not necessarily equal to transition times; differences between geminates could have been generated more quickly than divergence times based on the fossil record, and so such estimates represent maximum values. Other direct estimates of transition times have been made from the fossil record (summarized by Gould 2002: 852), ranging from 5,000 years for the Quaternary origin of dwarfed woolly mammoths on Wrangel Island, Alaska, to 73,000-275,000 years for the marginellid gastropod genus *Prunum*, to 100,000-200,000 years in a genus of Cretaceous marine ostracodes.

Transition times in the fossil record have also been discussed in the context of the theory of punctuated equilibrium (Eldredge and Gould 1972). Eldredge (1985: 189), for example, informally estimated that speciation usually takes "five to fifty thousand years". In attempting to define when a speciation event is "punctuated", as opposed to gradual, Gould repeatedly argued for a relative metric, such as 1-2 percent of the total subsequent duration of the species produced (Gould 1982, 2002: 768). Such a definition, he said, would allow "up to 100,000 years for the origin of a species with a subsequent life span of 10 million years" (the estimate for the average species of marine bivalves, Stanley 1979). Gould added, however, that he believed "that most events of speciation occur much more rapidly" (Gould 1982: 84).

Compilations of speciation intervals (Stanley 1979, Coyne and Orr 2004: 419) show that, although values range from very short (4,000 years for cichlid fishes in Lake Nabugabo, Uganda) to very long (several hundred million years for Notostracan crustaceans), most cluster between 1 and 20 million years. The compilation of Coyne and Orr (2004) yields a mean speciation interval for all taxa (protists to mammals) of 6.5 million years, with marine invertebrates showing higher values (most groups between 6 and 16 million years) than terrestrial plants, which in turn show longer intervals than terrestrial and freshwater insects and vertebrates. Marine gastropods show a mean value of 14.9 million years (Stanley 1979). Several more recent studies of divergence times of geminate pairs of marine gastropods across the Central American Isthmus indicate somewhat lower values (5-10 million years, which, as mentioned above, represent maximum values) (Collins 1996, Marko 2002).

Based on all of these data, the average lineage of marine gastropod appears to produce a new species roughly every 5-15 million years. Each of these new species, however, may actually arise in less than 100,000 years.

GEOLOGICAL ISSUES

The fossil record is incomplete (*e.g.*, Darwin 1859, Allmon 1989, 2001b, Valentine 1989, McKinney 1991, Foote 2001, Holland 2000, Kidwell and Holland 2002, Peters 2006, Smith 2007) because of a set of biological, geological, and taphonomic factors (referred to as "extrinsic biases" by Peters 2006). Our knowledge of the part of the record that *is* preserved is also incomplete (Signor and Lipps 1982, Marshall 2010) because of a set of sampling and taxonomic factors ("intrinsic biases", Peters 2006). Extracting biological signals from the fossil record therefore requires us to evaluate to what degree observed patterns in the fossil record are the result of biological changes, and to what degree the result of the structure of the stratigraphic record (*e.g.*, Kidwell and Flessa 1995, Holland 2000, Kidwell and Holland 2002, Peters 2006, Smith 2007).

The basic data for studying speciation in the fossil record consist of the distribution of appearances ("originations") of new taxa (usually referred to as "first appearance datums", or FADs, Van Couvering and Berggren 1977, Schoch 1989, Allmon 2003) in time and space. Because of the incompleteness of the record, however, an FAD for a taxon is in almost all cases later than—and in a different location from—the actual evolutionary first appearance ("EFA"). The greatest differences between FAD and EFA are likely to occur in association with unconformities—surfaces (representing intervals of time) of non-deposition and/or erosion. This has been called the "Unconformity Bias" of the fossil record (Holland 2000, Peters 2006). If FADs are coincident with an unconformity, then the time and/or place of the EFA may be highly uncertain because of the missing record (Jablonski 1980, Haq and Worsley 1982, Schoch 1989, Holland 2000). Indeed, the occurrence of multiple coincident FADs (and/or last appearances, or LADs) has been taken under some circumstances to be indicative of otherwise unrecognizable unconformities (Shaw 1964, Schoch 1989: 204). In such circumstances, a frequent assumption of paleontologists, "barring any evidence to the contrary, is generally that a FAD represents the immigration of a species shortly after its origination (evolution) in some unspecified area" (Schoch 1989: 201), but how "shortly after" and where that "unspecified area" was are usually left unstated. Thus, an FAD may be caused by a variety of frequently unknown phenomena, including *in situ* speciation or immigration, occurring at some unknown time (Fig. 1).

In the case of marine taxa, there is another important consideration. Major unconformities in marine stratigraphic sections are frequently the result of changes in sea level, which leads to non-deposition and/or erosion. Yet sea-level change is also one of the major environmental influences on benthic marine organisms on geological time scales, affecting habitat size and environmental conditions. In the context of the allopatric model of speciation, sea level change might theoretically cause cladogenesis by isolating populations of a species along a shallow shelf (Fig. 2, Valentine and Jablonski 1983, McKinney and Allmon 1995, Allmon *et al.* 1998). Sea level change is thus a major potential cause of both unconformities and evolutionary change (*e.g.*, Loutit *et al.* 1988, Patzkowsky and Holland 1993, Holland 1995, 2000, 2003, Allmon 2004, Allmon and Harries 2008). This has been called the "Common Cause" hypothesis (Peters and Foote 2002, Peters 2006).

In an analysis of all fossil genera of marine animals through the Phanerozoic, Peters (2006) found no significant correlation between mean duration of temporal hiatus at unconformities and genus origination rate (represented by FADs) in the following interval. Peters suggested that this result provides at least circumstantial support for the Common Cause hypothesis (such a conclusion would, of course, require close correlation between patterns of origination of genera and species, which might not be the case). The Common Cause phenomenon is likely a major issue in paleontological studies of speciation, since numerous studies, in a variety of taxa, have proposed connections between sea level change and species origination (*e.g.*, Hallam 1984, Bayer and McGhee 1985, Potts 1985, Rawson 1993, McKinney and Allmon 1995, McRoberts and Aberhan 1997, O'Dogherty *et al.* 2000, Sandoval *et al.* 2001, Zhang *et al.* 2006). Most of these studies, however, have not explicitly considered the potential effects of the Unconformity Bias.

Tools for dealing with the problem

The significance of a mismatch between a species' FAD and EFA thus depends on the "completeness" of its fossil record. A number of authors have attempted estimates of the overall completeness of the paleontological record (*e.g.*, Allmon 1989, Valentine 1989, McKinney 1991, Foote 1996,

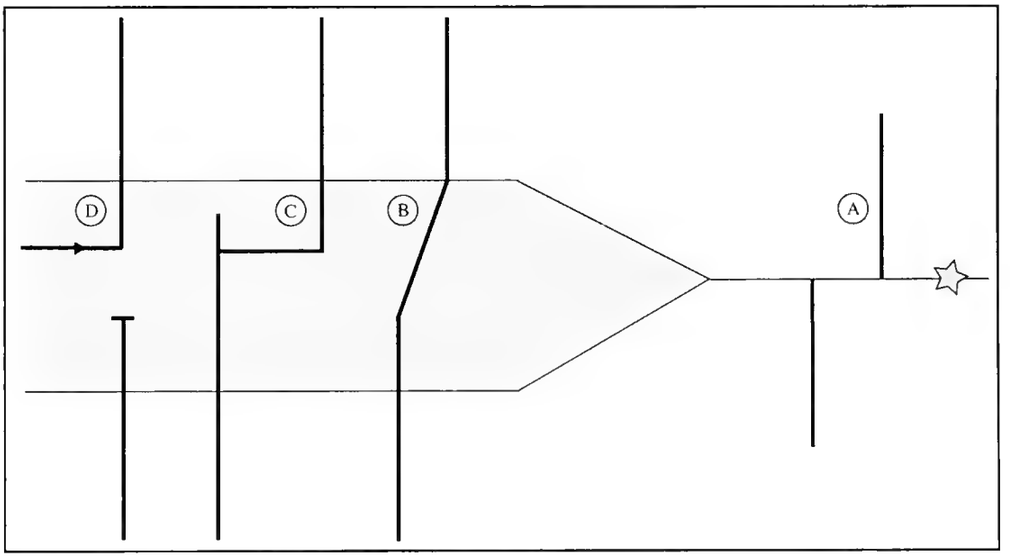

Figure 1. Schematic diagram showing three possible evolutionary events that may take place during a temporal hiatus (shading), coinciding with an unconformity (marked by the star). A, Observed pattern of an FAD immediately above the unconformity, coincident with the LAD of a potential ancestor immediately below the unconformity. This pattern could be caused by: B, anagenetic transformation of the older lineage into the younger lineage; C, cladogenetic event, producing the younger lineage as a daughter species of the older lineage, followed by extinction of the parent lineage; or D, extinction of the earlier lineage and invasion of the new lineage from outside the region.

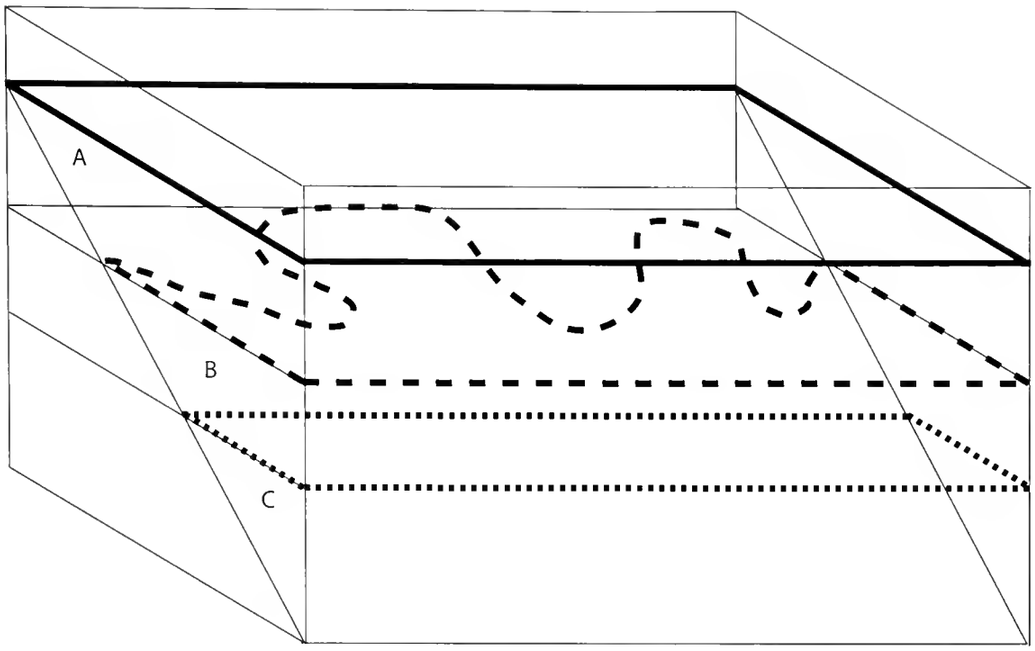

Figure 2. How change in sea level can cause allopatric speciation. As sea level falls from level A to level B, or rises from level C to level B, topographic irregularities on the shelf lead to formation of separate embayments or basins, which could isolate populations of a species.

1997, 2001, Foote and Sepkoski 1999, Benton *et al.* 2000). These techniques, however, are at too coarse a scale for the purposes described here. Others have developed techniques for estimating confidence limits for the stratigraphic range of a particular taxon (Marshall 2010 and references therein). These approaches are also not applicable to our situation because they cannot estimate missing ranges beyond the first and last recorded appearance of a taxon (Smith 2007).

We have therefore here developed four tests (Fig. 3) that can be applied to estimating *the confidence with which we can conclude that an observed FAD of a particular fossil species is roughly coincident with the actual time and place of the speciation event (EFA) that gave rise to that species.* The first of these tests takes a geographic perspective, attempting to estimate the likelihood that immigration explained an FAD. All other factors being equal, if no source of phylogenetically appropriate immigrants can be identified at roughly the same or slightly earlier time interval as an FAD, this will increase our confidence in equating the FAD with the time and place of the EFA. The other three take a temporal perspective, attempting to estimate how much time elapsed between a species' FAD and its EFA. All other factors being equal, the shorter the time, the greater our confidence could be in equating FAD and EFA.

Test #1: Potential sources of immigration

An FAD could record only *local* first appearance, *i.e.*, the species could have originated elsewhere and the FAD marks only the immigration event into the area represented by outcrops or collections (*e.g.*, Schankler 1981, Schoch 1989) (Fig. 3). The probability of such immigration can, in principle, be assessed if potential sources of immigration can be identified and analyzed for possible ancestors or sister species of the taxon showing the first appearance. This approach starts with specifying the "total potential source area" for a species, consisting of all the geographic areas that could potentially contribute ancestor/immigrants to explain a given FAD (*i.e.*, because of their distance, environment, etc.). In practice, this will be a list of all of the adjacent biogeographic units. For each of these areas, one can score (1) whether there are rocks of the appropriate age (*i.e.*, same as or just younger than the FAD), environment, and preservational potential, (2) whether there are faunal studies from these rocks, (3) whether these studies include likely candidate ancestor/immigrants, and (4) whether potential immigrants could reasonably have moved from the source to the site of the FAD, via some reasonable reconstruction of paleogeography, currents, etc.

This technique has been applied to similar problems before. Allmon (1990) and Vermeij (2001), for example, assessed the completeness of global biogeographic surveys of groups of Cretaceous and Cenozoic gastropods by compiling large lists of fossil faunas of appropriate age for the groups in question. In applying this approach (which Allmon (1990) called the "faunal survey" method), one must keep in mind that such lists can be biased by the more detailed and thorough investigation of certain groups or areas as compared to others, as well as the inherent problems of high-resolution intercontinental correlation. Webster *et al.* (2003) suggested that unpublished data from museum collections can also aid in this purpose.

Test #2: Ghost ranges

"Ghost range" is the term for the gap between a taxon's actual evolutionary origination and its appearance in the stratigraphic record, which can be inferred in cases in which phylogeny and stratigraphy are not completely congruent and sister taxa appear to originate at different times (Norell 1992, Benton and Storrs 1994, Wills 2007) (Fig. 3). When the phylogeny of the group is known, ghost ranges can be calculated to find an implied origination time older than the FAD. These implied originations may indicate a significantly different pattern than raw FAD data, suggesting a test for the completeness or adequacy of the record; all other factors being

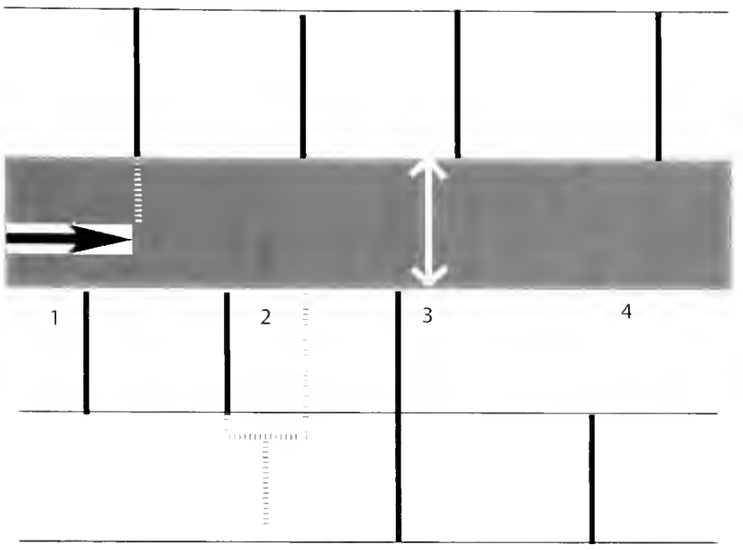

Figure 3. Four tests proposed here to evaluate the probability that a species' FAD (in stratigraphic unit 4) immediately above an unconformity approximates the time and place of that species' EFA. Test #1: Potential sources of immigration. What is the probability that the species immigrated rather than originated *in situ*? Test #2: Ghost ranges (dotted lines). What is the probability that the species originated before the hiatus marked by the unconformity? Test #3: Gap duration. What is the length of the hiatus? Test #4: Facies and sequence stratigraphy. Are the sedimentary environments below the unconformity likely to have been inhabited by the species? For example, the facies of unit 2 may never have contained the species, even though the species may have existed during this time, increasing our uncertainty that the species originated during the hiatus.

equal, shorter ghost ranges indicate a more complete stratigraphic record.

Test #3: Gap duration

Standard stratigraphic and geochronologic methods can be used to estimate the temporal duration of hiatuses at unconformities (*e.g.*, Allmon 1989, Paul 1998, Crampton *et al.* 2006b), and this can be compared to the observed duration of the taxon (Fig. 3). The ratio of the duration of the gap below a taxon's FAD (T) to the observed duration of the taxon (D) (which is of course also some function of its LAD) can be taken as a measure of the probability that a particular taxon actually originated at approximately the time and place of the FAD; lower ratios express higher probability.

Test #4: Facies and sequence stratigraphy

It is widely recognized that most marine fossil species tend to occur more commonly in some sedimentary facies than in others, and that much of this pattern reflects the ecological preferences of organisms (*e.g.*, Boucot 1981, Holland 2000). Many of the factors that control the geographic and environmental distribution of modern marine species are strongly correlated with water depth, which is also a major determinant of the structure of the stratigraphic record. That structure, as expressed in widely accepted stratigraphic models collectively known as "Sequence Stratigraphy" (Holland 2000, Coe *et al.* 2003), makes possible a set of predictions about the occurrence of fossils in outcrops (Holland 1995, 2000, Kidwell and Holland 2002) (Fig. 3). Under *constant* rates of origination and extinction, sequence stratigraphy predicts that:

Within a cycle of rise and fall in sea level, concentration of FADs (and LADs) is expected at the transgressive surface (TS, the point at which the shoreline migrates dramatically inland resulting in a rapid shift of depositional accommodation space shoreward, which in turn leads to lower sediment accumulation rates and even reworking on the shelf and formation of condensed beds offshore). The number of FADs at a TS should increase with both the degree of facies change across the surface and the temporal gap between the TS and the next oldest occurrence of the sedimentary facies immediately above the TS.

Concentrations of FADs (and LADs) are expected at sequence boundaries because of Unconformity Bias, and the number of FADs should increase with the length of the temporal hiatus at the sequence boundary.

Concentrations of FADs (and LADs) are expected in intervals of stratigraphic condensation (Holland 2000, Smith and Jeffery 2000). This Condensation Bias "alters perceptions of the relative timing of events, making events appear more closely spaced in time than they actually are" (Holland 2000: 157).

"Range offset", defined as the difference in age between the FAD of a species in a local stratigraphic section and its origination within the larger sedimentary basin, is expected to vary systematically within sequences, being large "within the transgressive systems tract of the deep basin [because of sediment starvation in the offshore as sea level rises], near the shelf break of the late highstand to early lowstand systems tract, and in extreme updip portions of shelves..." (Holland 2000: 157-158). ("Range offset" is equivalent to the difference between a species' FAD and its EFA if the EFA occurred in the same sedimentary basin.)

Based on these predictions, Holland (1995, 2000) suggested that the probability of finding each species in a stratigraphic section can be estimated by comparing its "preferred" facies to the distribution of that facies. In other words, the probability that a particular taxon actually occurred below its observed FAD can be estimated by whether that taxon would be expected to have occurred (and be preserved) in the sedimentary facies below that FAD. As illustrated in Fig. 3, if the facies immediately beneath an unconformity could reasonably be expected to have contained a species but does not, then we may have greater confidence that the observed FAD above that unconformity represents the approximate actual appearance of that species. If, on the other hand, the facies

immediately below the unconformity would not be expected to have contained or preserved the species had it existed at that time, then the actual evolutionary appearance of that species might have been significantly earlier.

Holland (1995, 2000) proposed a more detailed approach that could be applied either within or between facies, starting with making explicit estimates of three parameters for each species: peak abundance (PA), preferred depth (PD), and depth tolerance (DT), and then estimating paleo-depths through an outcrop. Our application here of the method to entire facies is justified because of the relative homogeneity within facies in the Coastal Plain Paleogene.

EXAMPLES FROM TURRITELLINE GASTROPODS

Turritelline gastropods (family Turritellidae, subfamily Turritellinae, *sensu* Marwick 1957a) are common components of many Early Cretaceous to Recent benthic marine assemblages worldwide (Allmon 1988, 2007), and frequently the most abundant macrofossils in assemblages in which they occur. They are taxonomically diverse, with more than 1800 described fossil and Recent species, appear to evolve relatively rapidly, and are among the most biostratigraphically important molluscan groups for their time interval; most species are (and probably were) largely sedentary suspension feeders, a mode of life unusual in caenogastropods (Allmon 1988, 2007). Here we use two recent but different analyses of species-level phylogenies in Late Cretaceous and Cenozoic turritellines from different parts of the world as case studies for application of the tests described above.

Paleogene of the U.S. Gulf and Atlantic Coastal Plains

A total of 55 turritelline species have been identified from Paleocene and Eocene sediments exposed on the Gulf and Atlantic Coastal Plains of the U.S. Allmon (1996) presented a phylogenetic analysis of 38 of these species (Fig. 4). Turritelline shells are morphologically relatively simple, with few discrete characters. This, together with the conclusion that the stratigraphic record of the Gulf and Atlantic Coastal Plain Paleocene and Eocene is relatively complete (Allmon 1989), justified the application of stratophenetic as well as purely cladistic techniques in phylogenetic analysis of turritellines from this interval (Allmon 1996).

The abundant, diverse, and well-preserved Paleogene mollusc faunas of the U.S. Gulf and Atlantic Coastal Plains have a long history of study (*e.g.*, Toulmin 1977). These faunas have long been known to exhibit a "stepped" or "punctuated" pattern of origination and extinction, in which blocks of relative faunal stability are interspersed with intervals in which as much as 90% of the fauna turns over, (*i.e.*, disappears and is replaced; Dockery 1984, 1986, Dockery and Lozouet 2003) even though the ecological changes at these transitions may not be nearly as profound (Sessa *et al.* 2010).

Test #1: Potential sources of immigration

Data available from Paleocene and Eocene mollusc faunas from the "total potential source area" of immigrants to the U.S. Gulf Coast (including West Africa (Adegoke 1977), Latin America (Allison and Adegoke 1969), California (Merriam 1941), and Europe (Cossmann 1912, Dockery 1984, Givens 1989)) suggest that some dispersal of marine gastropods, including turritellines, was indeed taking place during the Paleocene and Eocene (Allmon 1990). By comparing the taxa present in each of these potential source areas to Coastal Plain species at the time of their first appearance, it is possible to make a qualitative estimate of the probability that the Coastal Plain FADs represent immigration rather than true *in situ* origination. The results of this analysis (Allmon 1996) identified four instances (out of 38) in which immigration could plausibly be invoked (indicated by question marks in Fig. 4). Thus, all else being equal, this survey of potential sources of immigration suggests that most of these FADs probably represent in-situ origination.

Test #2: Ghost ranges

Because the phylogeny in Fig. 4 was not constructed using purely atemporal cladistics methods, recognition of ghost ranges in the strict sense of differences between a cladogram and observed stratigraphic ranges is not possible. Nevertheless, the phylogeny does permit some assessment of the completeness of inferred temporal durations of a number of species. For each species, we calculated a potentially "unpreserved" stratigraphic range as the duration between the observed FAD and the LAD of the most likely ancestor. The results indicate that 24 of 38 (63%) of the species have a potentially "unpreserved" range ("U") equal to or greater than their observed range ("O") (Table 3).

Test #3: Gap duration

The distribution of turritelline species in the Coastal Plain (Fig. 4) indicates that the majority (32 of 38, or 84%) of species show their first appearance immediately following hiatuses associated with cycles of sea level rise and fall. The duration of these hiatuses can be estimated and compared to observed species ranges. The results (Table 4) indicate that these gaps are all less than or equal to the mean observed species duration (3.6 million years, $\sigma = 0.5$).

Test #4: Facies and sequence stratigraphy

Following the approach of Holland (2000), we compared the occurrence of observed FADs of turritelline species at major unconformities in the Gulf Coastal Plain (Fig. 4) to the stratigraphic location of the next oldest sedimentary facies that might be expected to contain them (*i.e.*, based on their

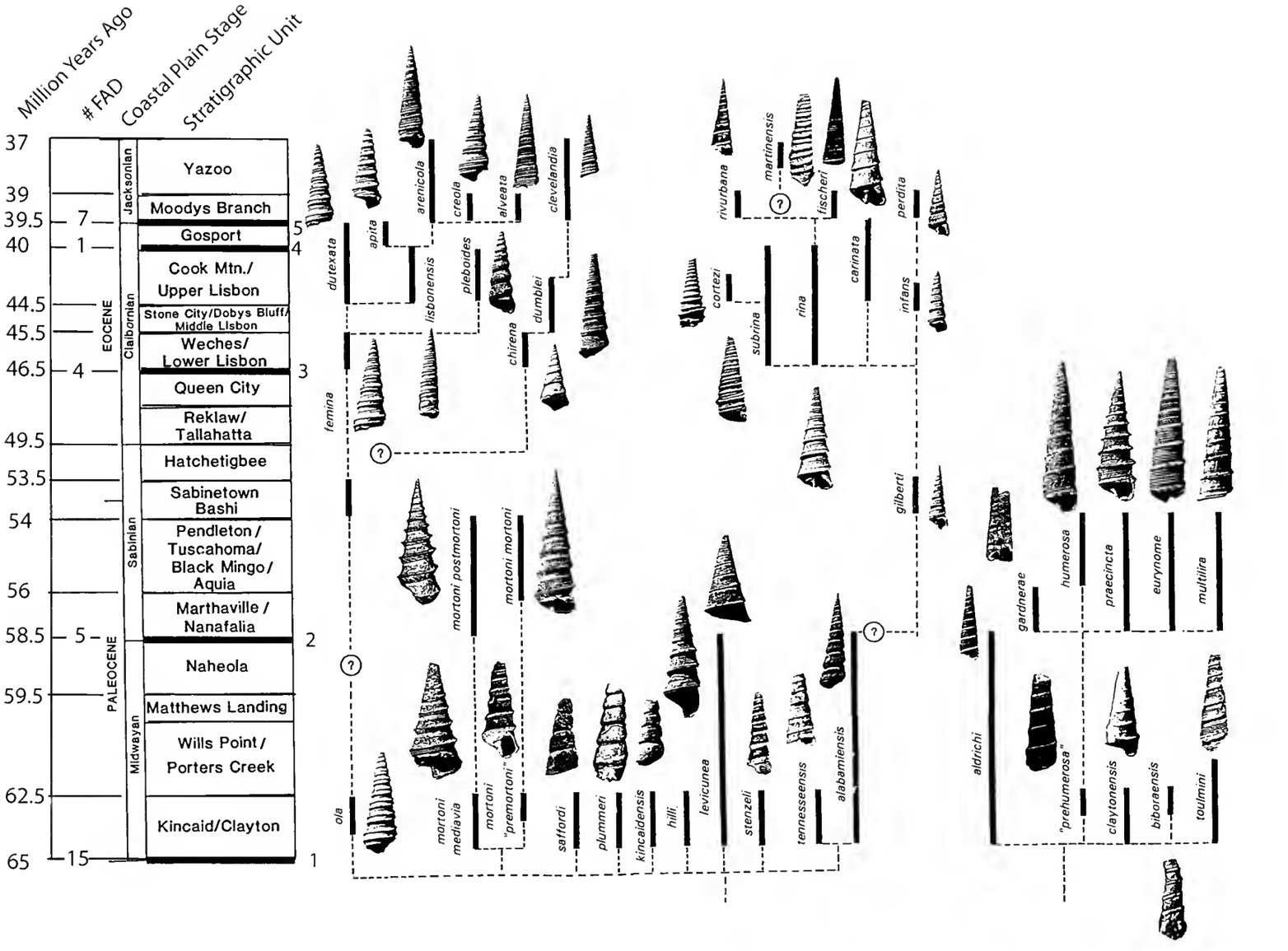

Figure 4. Phylogeny of turritelline gastropods from the Paleocene and Eocene of the U.S. Gulf and Atlantic Coastal Plains. Modified from Allmon (1996). Reproduced with permission.

observed pattern of facies occurrence). The results (Table 5) suggest that FADs at three of the five major unconformities occur in facies that lie directly on or very close to similar facies that lack the species (*i.e.*, have a high probability of representing the approximate EFA of their species). FADs at the other two major unconformities, in contrast, occur in facies that occur only well below the unconformity, and therefore have lower probability of approximating their respective EFAs.

Interpretation and application of results

Application of these four tests to the fossil record of Paleocene and Eocene turritelline gastropods of the U.S. Gulf and Atlantic Coastal Plains suggests that, although the overall stratigraphic record for this interval in this region is incomplete (Allmon 1989), a large proportion of the FADs of species in this clade appear to be relatively close to the EFAs, *i.e.*, are likely to be fairly reliable indicators of the approximate time and place of their actual evolutionary origination.

Although estimates of "unpreserved ranges" (analogous to ghost ranges) (Test #2, Table 3) suggests that more than half of the species have a potentially "unpreserved" range at least equal to their observed range, all of the estimated unpreserved ranges are less than half the length of the mean speciation interval for marine gastropods (14.9 million years) reported by Stanley (1979). The other three tests reflect even more favorably on the reliability of the record. The durations of the hiatuses associated with the five major unconformities are all less than or approximately equal to the mean observed species duration (3.6 million years) (Test #3, Table 4) (and

Table 3. Observed durations for the turritelline species shown (Fig. 4), compared to estimated unpreserved duration (see text for discussion). Observed durations modified from Allmon (1996), using numerical dates of stratigraphic units mostly from Baum and Vail (1988).

Species	Observed duration (my) (O)	"Unpreserved range" (my) (U)	Ratio U:O
alabamiensis	6.5	3.5 [4]	0.54
aldrichi	6.5	3.5	0.54
alveata	0.5	0.5	1.00
apita	0.5	0	0.00
arenicola	2.5	0.5	0.20
carinata	5.0	0	0.00
chirena	1.0	7.5	7.50
claytonensis	2.5	3.5	1.40
clevelandia	2.5	4.5	1.80
cortezi	4.5	0	0.00
creola	0.5	0.5	1.00
dumblei	1.5	0	0.00
dutexata	5.0	1.0	0.20
eurynome	4.5	4.5	1.00
femina	8.5	8.5	1.00
gardnerae	2.5	4.5	1.80
gilberti	0.5	4.5	9.00
hilli	2.5	3.5	1.40
humerosa	2.0	7.0	3.50
infans	1.0	0	0.00
kincaidensis	2.5	3.5	1.40
lisbonensis	4.5	1.0	0.22
levicunea	6.5	3.5	0.54
mortoni mortoni [1]	10	4.5	0.45
mortoni posmortoni [2]	11	3.5	0.32
multilira	4.5	4.5	1.00
ola	2.5	5.5	2.20
perdita	0.5	4.5	9.00
pleboides	4.5	1.0	0.22
plummeri	2.5	3.5	1.40
praecincta [3]	4.5	4.5	1.00
rina	6.5	7.0	1.08
rivurbana	0.5	0.5	1.00
saffordi	2.5	3.5	1.40
stenzeli	2.5	3.5	1.40
subrina	6.5	7.0	1.08
tennesseensis	2.5	3.5	1.40
toulmini	2.5	3.5	1.40

[1] Allmon (1996) interpreted *Kapalmerella mortoni mortoni* as the direct anagenetic descendant of *K. mortoni premortoni*, thus the "observed" duration given here is the total from the FAD of *K. m. premortoni* to the LAD of *K. m. mortoni*.

[2] Allmon (1996) interpreted *Kapalmerella mortoni postmortoni* as the direct anagenetic descendant of *K. mortoni mediavia*, thus the "observed" duration given here is the total from the FAD of *K. m. premortoni* to the LAD of *K. m. mortoni*.

[3] Allmon (1996) recognized two subspecies of "*Turritella*" *praecincta*: "*T.*" *p. praecincta* from Alabama (with an observed duration of 4.5 my) and "*T.*" *p. virginiana* from Maryland and Virginia (with an observed duration of 2.0 my). The longer of the two is given here.

[4] The "unobserved" durations of all species in the Kincaid/Clayton is given as the estimated temporal gap across the Cretaceous-Tertiary boundary in the Gulf Coastal Plain (*i.e.*, between the Kincaid/Clayton and the Owl Creek/Prairie Bluff), 3.5 million years (Hazel *et al.* 1984).

again all are smaller than the mean speciation interval for marine gastropods). Similarly, analysis of the stratigraphic pattern of facies likely to contain fossils of these species above and below these unconformities (Test #4, Table 5) suggests that these patterns produce uncertainties greater than mean species durations at only two of the five major unconformities. Analysis of potential sources of immigration of these species (Test #1), furthermore, suggests that 90% of the first appearances have a high likelihood of being *in-situ* evolutionary events rather than immigration events.

This analysis does not mean that we can read the fossil record of these gastropods literally, and treat all first appearances as true evolutionary events. It does suggest, however, that these patterns of geological first appearances should not be wholly discounted as a source of evolutionary insight. Most (84%) of the species in Fig. 4 show their first appearances during hiatuses associated with five major unconformities. If we have reasonable confidence that this represents the actual time and place for most of these species, this suggests support for the "Common Cause" hypothesis (Peters and Foote 2002, Peters 2006). The association of first appearances of species with unconformities resulting from cycles of rising and falling sea level might, in other words, be telling us that transgressions and regressions of the shoreline (Fig. 2) could have played a significant role in the evolution of this group.

There is a long history of connecting faunal and sea-level changes in the Coastal Plain (*e.g.*, Palmer 1979, Baum and Vail 1988, Loutit *et al.* 1988). Dockery (1984, 1986), for example, argued that sea-level changes are primarily responsible for high faunal turnover in the Coastal Plain Paleogene, whereas Hansen (1987, 1992) argued for temperature changes (cooling) or extraterrestrial impact as the primary cause (Hansen 1987, Allmon and Ivany 2008, Sessa *et al.* 2010). Our results thus offer at least circumstantial support for sea-level as an important causal factor in speciation.

Table 4. Estimated duration of the five major unconformities shown in Fig. 4.

Unconformity	Duration (millions of years)	Notes
5	1.0?	(5)
4	0.6	(4)
3	<0.5?	(3)
2	1.0	(2)
1	3.5	(1)

(1) Cretaceous-Tertiary boundary: Hazel *et al.* (1984)

(2) Naheola-Nanafalia contact: Hazel *et al.* (1984)

(3) Tallahatta-Lisbon contact: Stenzel (1952) suggested that there was a significant hiatus between the Tallahatta and the Lower Lisbon in Alabama, but Hazel *et al.* (1984) disagreed. In Texas, the Tallahatta equivalent is a deltaic unit (the Queen City Formation) which interfingers with a fossiliferous marine unit (the Reklaw Formation). It is therefore possible that there was a minor hiatus at this time.

(4) Lisbon-Gosport contact: Hazel *et al.* (1984)

(5) Gosport-Moodys Brach contact: The contact between the Gosport and Moody's is clearly and sharply unconformable (*e.g.*, Dockery 1986), but a precise estimate of the length of the hiatus is difficult to obtain, in part because it was likely very brief. Both the Gosport Sand and the Moody's Branch Formation were placed in zone NP 17 by Siesser (1983) based on calcareous nannofossils. Zone NP17 has a duration of approx, 3.0 million years (*e.g.*, Staerker 1998). The time necessary for formation of these units is also unknown, but likely to have been brief; here we have used a figure of 0.5 my for each.

Cenozoic of New Zealand

The New Zealand Cenozoic mollusc record is one of the most abundant, diverse, and well studied in the world. The faunas have been extensively studied taxonomically (*e.g.*, Marwick 1929, Laws 1934a, 1934b, 1940, Fleming 1955, Beu 1970, Maxwell 1978, 1992, Beu and Maxwell 1990, Stillwell 1993, Beu 2004, 2006), and used for paleoenvironmental reconstruction (*e.g.*, Fleming 1944, Beu 1974), biostratigraphy (*e.g.*, Beu 1969, Cooper *et al.* 2001, Cooper 2004), and examinations of diversity patterns (*e.g.*, Beu 1990, Crampton *et al.* 2003, 2006a, Hendy 2007, Hendy *et al.* 2009).

Turritellines are an important component of the New Zealand Cenozoic mollusc record (*e.g.*, Marwick 1957a, 1957b, 1971, Beu 2010), with nine genera occurring through the Cenozoic. Three of these genera (*Zeacolpus* [Finlay, 1926], *Stiracolpus* [Finlay, 1926], and *Maoricolpus* [Finlay, 1926]) also have species still extant around the coast of New Zealand. As with turritellines of other geographic regions (Allmon 2007), New Zealand fossil and Recent turritellines are often found in high abundances (*e.g.*, Grace and Hayward 1980, Hayward *et al.* 1981), and are ecologically important components of the faunas in which they are found.

Three peaks of FADs have been observed in the total New Zealand Cenozoic molluscan fauna (Beu 1990, Beu and Maxwell 1990): the Middle Eocene, Middle Oligocene, and Early Pliocene. These FAD peaks are not highly correlated with standing diversity of the molluscan fauna (Beu 1990, Crampton *et al.* 2006a, Hendy 2007), which peaked in the Middle Miocene, but there are increases in diversity at these times when compared to the stages immediately before and after.

The Cenozoic turritelline fauna of New Zealand includes approx. 60 species (Beu 2010, Smith 2011). The FADs of these species show a generally constant low level through the Cenozoic, from the first appearance of the subfamily in the Paleocene. The two most species-rich New Zealand

Table 5. Sequence stratigraphic analysis of completeness of the fossil record of turritelline gastropods in the Paleocene and Eocene of the U.S. Gulf Coastal Plain (based on the phylogeny and species ranges shown in Fig. 4).

Unconformity	Overlying stratigraphic unit containing FAD	Number of species FADs	Next oldest similar facies	Mean temporal gap (my) between FAD and next oldest similar facies
5	Moodys Branch	7	Gosport [1]	≅ 0
4	Gosport	1	Upper Lisbon/ Cook Mtn. [2]	≅ 0
3	Weches/Lower Lisbon	4	Reklaw/ Bashi [3]	3.0-7.0
2	Nanafalia	5	Naheola/ Matthews Landing [4]	< 1.0
1	Kincaid/Clayton	15	Owl Creek/ Prairie Bluff [5]	4.0

[1] Both the Gosport and Moody's Branch are glauconitic, high-density shell beds (Toulmin 1977, Baum and Vail 1988).

[2] Although they are not glauconitic, high-density shell beds like the Gosport, the Lisbon and Cook Mountain formations are highly fossiliferous and contain a high species diversity of molluscs, including many in common with the Gosport (Toulmin 1977, Dockery 1980), suggesting similar preservation potential.

[3] The Reklaw Formation of Texas contains a low density of fossils, but a very high diversity of species represented by very well-preserved specimens (Garvie 1996). The correlative Tallahatta Formation of Alabama and Mississippi, however, has only rare and very poorly-preserved fossil molluscs. The next oldest comparable facies to the Lower Lisbon in the eastern Gulf region if the Bashi Formation.

[4] Although the Naheola Formation of Alabama does contain fossils of some species in common with the overlying Nanafalia, not all are well-preserved, suggesting some difference in preservation potential. The Matthews Landing beds, however, have an extremely well-preserved and very diverse molluscs fauna.

[5] The Owl Creek and Prairie Bluff formations of Tennessee, Mississippi, and Alabama contain occasional shell beds with diverse, abundant, and well-preserved molluscs (Sohl 1960, Mancini *et al.* 1995).

turritelline genera are *Zeacolpus* (23 species) and *Stiracolpus* (30 species), whose FADs show a pattern approximately the same as the group as a whole, from the appearance of *Zeacolpus* in the Middle Eocene, with smaller peaks of FADs in the Miocene (*Zeacolpus*) and Pliocene (*Stiracolpus*) (Fig. 5). A recent phylogenetic analysis of *Stiracolpus* and *Zeacolpus* (Smith 2011) (Fig. 6) makes possible a more detailed analysis of this record.

Test #1: Potential sources of immigration

Peaks of FADs in both *Zeacolpus* and *Stiracolpus* occur in the first stages in which they both occur—the Bortonian (Middle Eocene) for *Zeacolpus* and Opoitian (earliest Pliocene) for *Stiracolpus* (Fig. 5). This is also where there are peaks in EFAs based on ghost ranges (see below) that occur in both genera (though part of this pattern may be an artifact due to the age of the outgroup used in calculating the phylogeny [Smith 2011]). Both of these stages also show peaks of first appearances in the rest of the molluscan fauna (Beu and Maxwell 1990), and intervals in which calculated origination rate (see below) is high (Crampton *et al.* 2006a). The first of these, the Middle Eocene Bortonian peak in total molluscan FADs, has previously been explained as due to immigration of much of the fauna, including *Zeacolpus*, into New Zealand at that time (Beu and Maxwell 1990), though the source of the immigrants is not clear. Part of the high rates of total molluscan FADs in the Middle Eocene Bortonian stage may also be due to the relatively long duration of that stage (Crampton *et al.* 2006b).

The peak in FADs in *Stiracolpus* at the base of the Pliocene corresponds with the appearance of a previously recognized

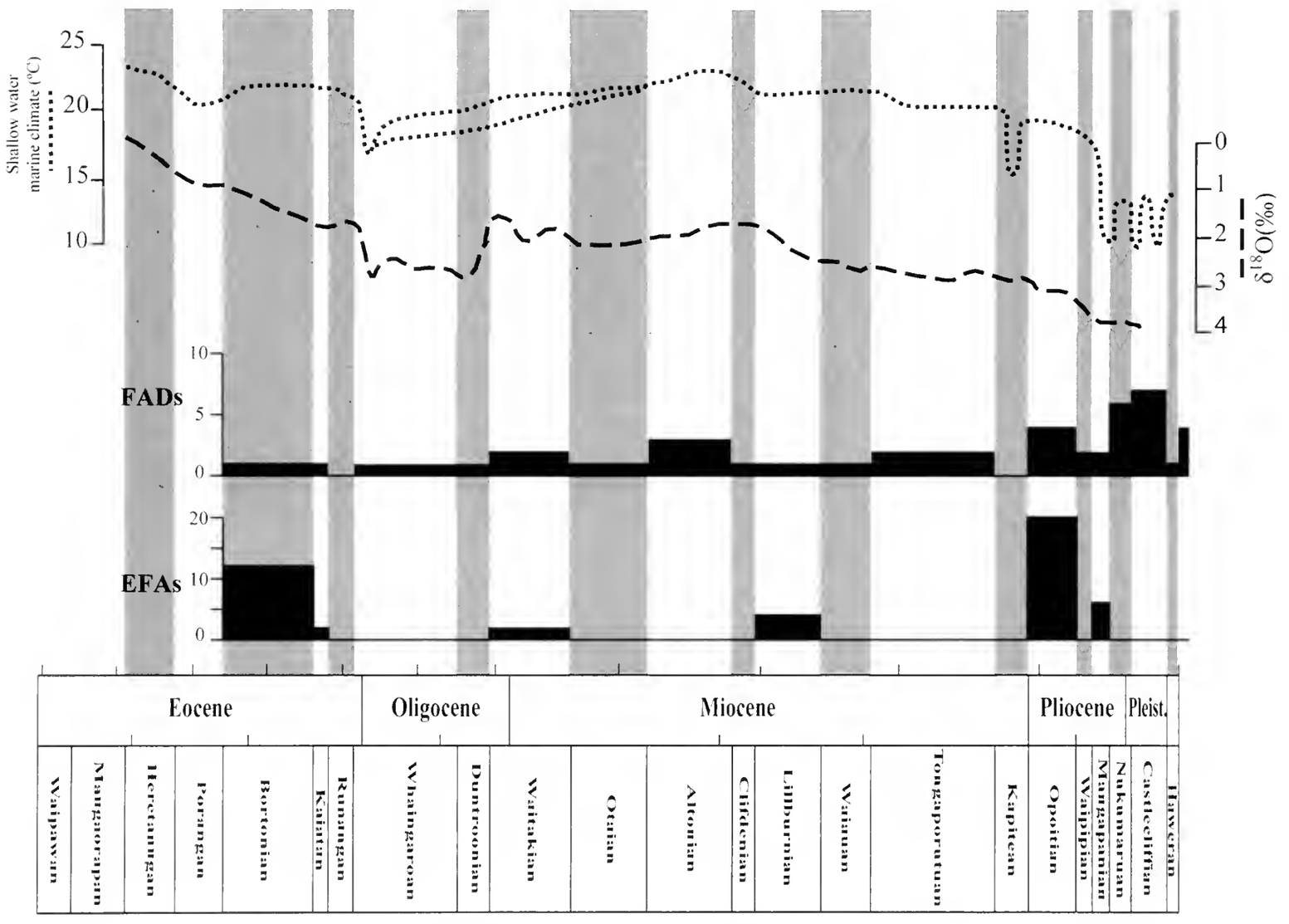

Figure 5. Number of FADs of *Stiracolpus* and *Zeacolpus* species per stage in the New Zealand Cenozoic from the first appearance of *Zeacolpus* in the middle Eocene to Recent, and number of calculated originations (EFAs) per stage for *Stiracolpus* and *Zeacolpus* based on ghost ranges implied by a phylogenetic analysis of each genus (Fig. 6). Shallow water marine temperature (dotted line) adapted from Hornibrook (1992), $\delta^{18}O$‰ curve (dashed line) adapted from Zachos *et al.* (2001).

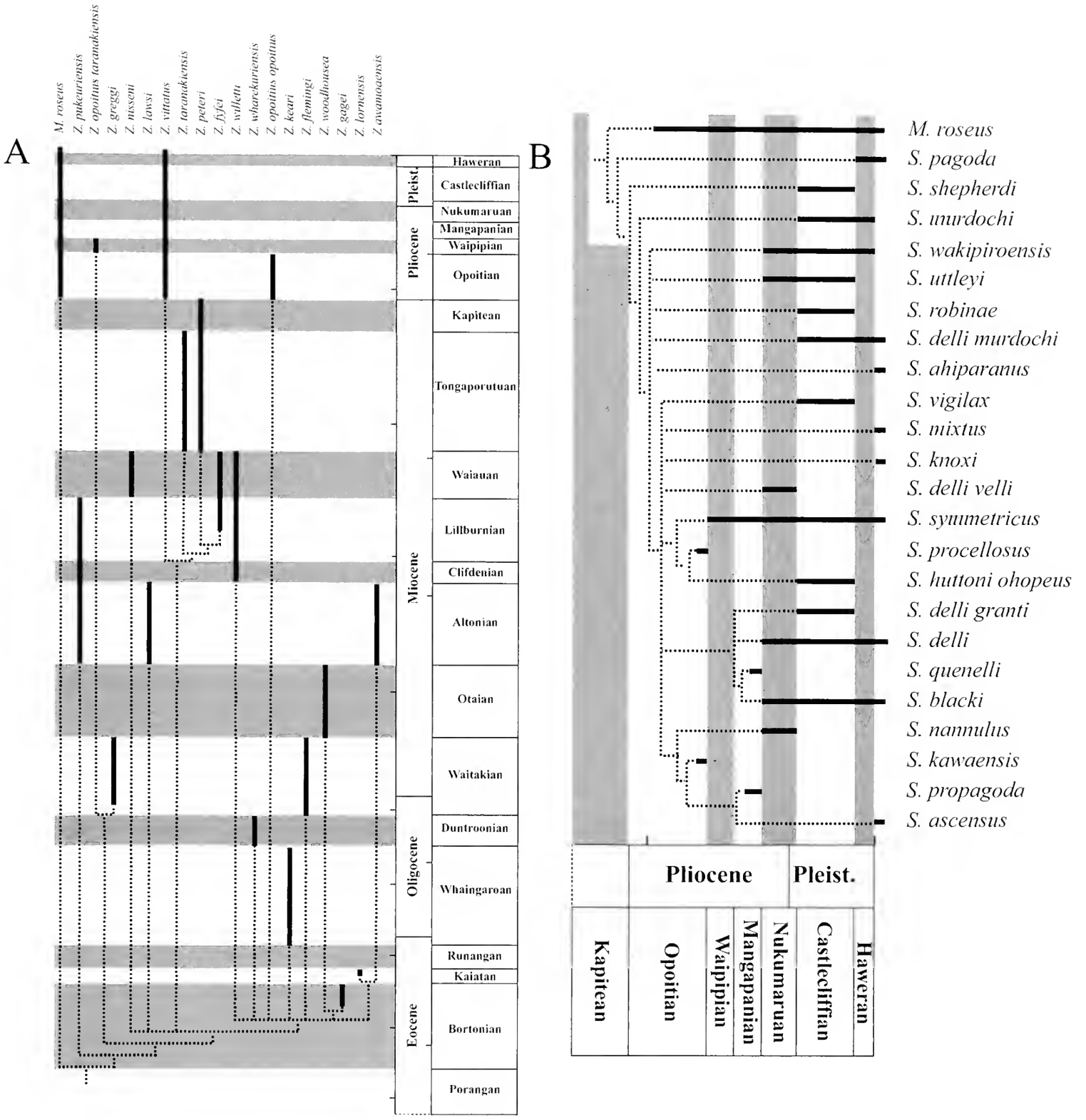

Figure 6. Phylogenies of *Zeacolpus* (A) and *Stiracolpus* (B) from Smith (2011), plotted on the New Zealand time scale's stages (Cooper 2004). Both phylogenies are frequency difference consensus trees from phylogenies generated under a number of different weighting schemes using both traditional characters (from protoconch and teleoconch) and characters from a geometric morphometric analysis. Solid black lines indicate the stages from which a species has been reported but do not necessarily imply that species is present through the entire stage. Dotted lines indicate implied ghost range inferred from phylogeny. Species ranges have occasionally been shortened to not range through an entire stage in order to accommodate branching events inferred to occur within that stage. Such "shortened" ranges do not imply that species occurred only in the later part of that stage.

cooler-water fauna that began to appear in New Zealand at the end of the Miocene, presumably due to intensification of the circum-Antarctic current (Fleming 1965, Beu 1990, Beu *et al.* 1997) and in conjunction with a major ecological turnover (Hendy *et al.* 2009). The majority of *Stiracolpus* species from this interval are geographically restricted, and it is therefore possible that some species could have immigrated from other basins elsewhere in New Zealand. For instance, Beu (2004) shows that there are occurrences of warm-water species within the Wanganui Basin (where the majority of *Stiracolpus* species are found) in the south-west of the North Island, which probably originated in the north-east of the North Island and were intermittently transported as larvae through temporary seaways.

Although immigration is a likely explanation of the generic first appearance of both *Zeacolpus* and *Stiracolpus* in New Zealand, it seems less likely that multiple species of each genus immigrated at the same time; in the case of *Zeacolpus* the phylogeny in Fig. 6 suggests at a minimum 10 species, and in that of *Stiracolpus*, 12 species. Speciation *in situ* following immigration seems a more likely explanation simply from a probabilistic point of view. Immigration into New Zealand has evidently occurred in other genera of turritellines at other times, *e.g.*, *Maoricolpus* appears to have originated in Australia and transferred to New Zealand, later becoming extinct in Australia while in New Zealand it survived until the Recent (*e.g.*, Allmon *et al.* 1994).

Test #2: Ghost ranges

Cladistic phylogenies of the two genera *Zeacolpus* and *Stiracolpus* (Smith 2011) imply ghost ranges for every included species of both genera (Fig. 6). The originations implied by these phylogenies show a very different pattern than the raw FADs for both genera (Fig. 7), with originations occurring in bursts, rather than gradually through time, and with considerable ghost ranges being implied for nearly every species. (These bursts of inferred EFAs are partially due to the choice of outgroup in the phylogeny—a different outgroup with an older stratigraphic range may have elongated the ghost ranges even further [Smith 2011] but there is only one New Zealand turritellid species that occurs before the oldest *Zeacolpus* species in the analysis, and therefore this conservative selection of outgroup seems justified.) U:O ratios (Table 6) indicate unpreserved ranges greater than the observed ranges in nearly all cases. In *Zeacolpus* the mean U:O value is 6.32 while in *Stiracolpus* it is 80.47 (although if species only observed in the Recent are excluded, this drops dramatically to 2.37). It is important to note that the observed ranges used to calculate these ratios are taken as spanning the full length of any stage in which the species is found, making O in all cases likely larger than was actually the case. These ratios are thus actually conservative estimates.

The ghost ranges calculated are long, but not exceptionally so for marine invertebrates (*e.g.*, Wills 2007 and references therein). The maximum range extension implied is approx. 35 million years in *Zeacolpus*, but in the pre-Pliocene record of New Zealand, such range extensions are not implausible, as fossil occurrences are usually highly localized. In *Stiracolpus*, the ghost ranges implied by the phylogeny are up to ~6 million years for a species known only from the Recent. Although such ranges may not be unusual for most groups, it is almost the entirety of the total stratigraphic range of the species in New Zealand, implying that there is an extensive gap in the fossil record of this genus. Given the resolution and highly fossiliferous nature of the majority of the beds of the New Zealand Pliocene and Pleistocene basins, ghost ranges extending through the majority of the Plio-Pleistocene would appear to be unlikely. Immigration from other basins (*e.g.*, Beu 2004) could account for some of the ghost ranges but their extent and ubiquity suggests immigration cannot explain them all.

Test #3: Gap duration

In order to assess gap duration it is necessary to know not just the duration of the species and the duration of the hiatus during which it may have originated, but also to what degree a hiatus may be directly associated with a particular FAD (*i.e.*, does the FAD occur immediately above the hiatus, or somewhere within the overlying facies). This requires a level of stratigraphic resolution sufficient to determine the duration between an FAD and an underlying unconformity. This level of resolution, however, is unfortunately not available for the majority of *Zeacolpus* species, and so we cannot directly associate hiatuses with FADs or calculate hiatus length. Another approach to analysis of *Stiracolpus*, however, may be more profitable.

Stiracolpus occurs in the Pliocene and Pleistocene, the stratigraphic record of which in New Zealand is much better resolved than the earlier stage of the Cenozoic. This portion of the stratigraphic record is one of the most complete in the world (*e.g.*, Naish *et al.* 1998, Saul *et al.* 1999) with very little time being unrepresented, mostly hiatuses within cyclothems in the stratigraphic sequences. Values for T can therefore be much better constrained in this stage than earlier, as it consists of well-dated and well-constrained cyclothems corresponding to the 41,000 and 100,000 year Milankovitch cycles (Abbott 1998, Carter and Naish 1998). A conservative estimate of hiatus length within one of these cyclothems might be 10,000 years, which is almost certainly an overestimate given the completeness of the sequences and lack of missing material and time in the cyclothem sequences (Abbott 1998). Using this estimate, however, we can calculate observed durations (D) for species of *Stiracolpus*. (There are multiple other sources of error in this calculation; for instance, D will be an

Table 6. Observed durations for the New Zealand fossil species in the genera *Stiracolpus* and *Zeacolpus* shown (Fig. 6), compared to estimated unpreserved duration. Data from Smith (2011).

Species	Observed duration (my) (O)	"Unpreserved range" (my) (U)	Ratio U:O
S. ahiparauus	0.01	4.51	451.00
S. ascensus	0.01	3.00	300.00
S. blacki	2.41	0.77	0.32
S. delli	2.41	0.60	0.25
S. delli granti	1.29	1.37	1.06
S. delli murdochi	1.64	2.88	1.76
S. delli velli	0.77	2.88	3.74
S. huttoni oliopeus	1.29	3.65	2.83
S. kawaensis	1.68	0.00	0.00
S. knoxi	0.01	5.28	528.00
S. mixtus	0.01	5.28	528.00
S. murdochi	1.63	3.65	2.24
S. nannulus	0.77	2.88	3.74
S. pagoda	0.35	6.16	17.60
S. procellosus	1.68	0.00	0.00
S. propagoda	0.60	0.00	0.00
S. queuelli	0.60	0.00	0.00
S. robinae	1.29	2.88	2.23
S. shepherdi	1.29	3.65	2.83
S. symmetricus	3.61	1.68	0.47
S. uttleyi	2.06	2.88	1.40
S. vigilax	1.29	2.88	2.23
S. waikipiroensis	2.40	2.88	1.20
Z. awamoaensis	3.10	18.00	5.81
Z. fleming	3.50	17.80	5.09
Z. fyfei	4.18	0.00	0.00
Z. gagei	6.00	0.00	0.00
Z. greggi	3.50	0.00	0.00
Z. keari	7.00	8.70	1.24
Z. lawsi	3.10	27.10	8.74
Z. lorneusis	1.00	0.00	0.00
Z. uisseni	1.78	30.30	17.02
Z. opoitius opoitius	1.68	40.62	24.18
Z. opoitius tarauakiensis	1.68	39.40	23.45
Z. peteri	5.64	4.18	0.74
Z. pukeuriensis	6.30	24.00	3.81
Z. tarauakiensis	4.42	4.18	0.95
Z. vittatus	5.28	9.82	1.86
Z. wharekuriensis	2.10	15.70	7.48
Z. willetti	4.98	27.10	5.44
Z. woodhousea	2.70	21.30	7.89

[1] Species observed only in the Recent are assumed to have an observed range (O) of 10,000 years.

[2] *Stiracolpus murdochi* used here is an unpublished species described by Marwick in the GNS collections.

overestimate as total species duration is calculated by summing the total length of any stage in which that species occurs.) Calculated this way, the average D of *Stiracolpus* species is 1.27 million years—orders of magnitude greater than a 10,000 year hiatus. These figures could be better constrained in the future given the level of stratigraphic resolution available in the Plio-Pleistocene basins of New Zealand.

A more precise estimate can be made for the seven species of *Stiracolpus* that have FADs in the Castlecliffian stage (approximately equivalent to the start of the Pleistocene—the Plio-Pleistocene boundary actually falls towards the end of the previous stage, the Nukumuruan [Cooper 2004]). At the unconformity between the Nukumuruan and the Castlecliffian stages there is a 500,000 year hiatus (Abbott *et al.* 2005). Of the seven species of *Stiracolpus* having FADs at in the Castlecliffian stage, five are known only from the Castlecliffian (stage length = 1.29 million years), one of which extends into the Haweran (stage length = 0.34 million years) and one of which is present in the Recent. The average duration (D) of these species is 1.76 million years, which is approximately 3.5 times longer than the length of the hiatus (T) of 0.5 million years.

Test #4: Facies and sequence stratigraphy

The patchy geographic distribution and low temporal resolution of much of the pre-Pliocene strata of the New Zealand Cenozoic make a sequence stratigraphic approach difficult, as it requires correlation of co-temporal stratigraphic events at a higher resolution than currently possible. New Zealand has been tectonically active during most of the Cenozoic, and calculating local sea level variation is thus very difficult. There is therefore not a well-constrained sea level curve available for the majority of the New Zealand Cenozoic (Crampton *et al.* 2006a). However, five second-order sequence stratigraphic cycles—*i.e.*, cycles influenced only by tectonics rather than eustasy—have been identified from the Eocene to the Recent (King *et al.* 1999, Crampton *et al.* 2006b). Although a widespread transgression in the Bortonian has been identified (Beu and Maxwell 1990), which could be associated with an apparent peak in

speciation in *Zeacolpus* during this stage, it is harder to identify sea level variations that correspond to the other peaks in FADs or calculated origination in either genus. In cases in which other peaks do correspond with some variation in sea level, it is not necessarily a drop (for instance, the peak in the Waitakian [Early Oligocene] actually corresponds with the period of maximum flooding of New Zealand [Wilson 1956, Crampton *et al.* 2006b: fig. 6] during a first order sequence stratigraphic cycle rather than a drop in sea level).

Crampton *et al.* (2006b) have shown that sampling probability in the New Zealand Cenozoic molluscan shelf fauna is linked to the second order sequence stratigraphic cycles, with highest sampling probability occurring during mid-cycle, due to enhanced preservation potential during these periods in addition to erosional loss at sequence boundaries. These peaks in sampling probability coincide with peaks in FADs in *Zeacolpus* and *Stiracolpus* (but only the two in the Middle Eocene and Lower Pliocene also correspond with peaks in calculated originations). The high number of FADs in these stages may be at least partially a sampling artifact due to low sampling intensity in prior stages. The Late Miocene (immediately prior to the first appearance of *Stiracolpus*) contains a high proportion of bathyal rather than shelf facies in which molluscs are less frequent, whereas the Paleocene to Early Eocene (prior to the appearance of *Zeacolpus*) is poorly represented in the New Zealand rock record. Other genera of turritellines have their FADs in the same stage as *Zeacolpus* (*e.g.*, *Amplicolpus*) but the previous turritelline occurrence prior to this stage occurred in the Lower Paleocene, approx. 20-25 my previously. It is, therefore, theoretically possible that the EFA of *Zeacolpus* could be up to 25 My before the first *Zeacolpus* FAD.

As mentioned above, however, the Pliocene and Pleistocene basins of New Zealand consist of stacked series of cyclic shallow-marine strata (*e.g.*, Naish *et al.* 1998, Saul *et al.* 1999). These sequences are well exposed on land (due to uplift along the boundary of the Pacific and Australian plates (Naish 2005), and have been intensively studied since Fleming (1953) first described the fossiliferous sections of the Wanganui Basin (*e.g.*, Vella 1963, Beu and Edwards 1984, Kamp and Turner 1990, Abbott and Carter 1994, 1999, Pillans *et al.* 1994, Naish *et al.* 1998, 2005a, 2005b, Saul *et al.* 1999, Abbott *et al.* 2005, Turner *et al.* 2005). Although detailed bed-level analyses have not yet been carried out on the New Zealand turritellines to compare FADs and ghost ranges horizon-by-horizon through the Plio-Pleistocene, this is an area where the techniques used here could be profitably employed.

Interpretation and application of results

The records of turritellines in the Paleogene of the U.S. Coastal Plain and the New Zealand Cenozoic show important differences, both in the temporal resolution of the record and (to the degree that conclusions are possible) in apparent evolutionary responses to environmental variation. Although peaks of FADs for *Zeacolpus* do correspond with periods of high sampling probability (*sensu* Crampton *et al.* 2006a, 2006b), taken as a whole the pre-Pliocene fossil record of turritellines in New Zealand appears to be of only limited utility for drawing useful conclusions about the evolutionary significance of observed FADs. The Plio-Pleistocene record, however, appears to show more potential.

Test 1 suggests that *Zeacolpus* immigrated into New Zealand from elsewhere, and then probably speciated *in situ* afterwards. The under-sampling of the stages immediately prior to the appearance of each genus means that this speciation may not have occurred within a single stage, but could have happened over a longer time period. This is particularly true in the case of *Zeacolpus*, where there are multiple poorly represented stages prior to the first appearance of the genus in the Bortonian. The very long ghost ranges of test 2 also suggest that the stratigraphic record is very incomplete. Many of these ghost ranges pass through stages that have a high sampling probability (Crampton *et al.* 2006a), in which it would be expected that fossils would be found if the species was present. The geographic patchiness of the pre-Miocene record, however, could mean that exposure is not available in the areas in which the species might be expected even if the correct time period is sampled.

This is less likely to be the case in the Pliocene and Pleistocene. Although tests 1 and 2 suggest that FADs of *Zeacolpus* and *Stiracolpus* may not be accurate representations of when species actually originated, test 3 is more hopeful at least in the case of *Stiracolpus* where hiatuses in the stratigraphy of the basins are very short compared to the duration of species. The hiatus at the base of the Castlecliffian stage of 500,000 years, while longer than others in these sequences, is still short compared to species duration and is of a magnitude in which speciation is observed in other species. While hiatuses underlying FADs in *Zeacolpus* may also be of similar magnitude, it has not been possible to test this here.

EFAs and, to a lesser extent, FADs in the two turritelline genera here appear to show some correlation with cool periods (Fig. 5). Though some of the peaks in both FADs and EFAs may be artifactual (*e.g.*, due to poor sampling in previous stages), this cooling pattern appears to be the only consistently identifiable factor associated with periods of high origination in these two genera. Decreases in temperature have normally been associated with extinction events rather than speciation (*e.g.*, Hansen 1987, 1988), but decreasing temperature could also lead to allopatic speciation through vicariance (*e.g.*, Evans *et al.* 2005) in a similar way as sea level fall (Fig. 2). In the case of either temperature or sea level change being correlated with inferred speciation in *Stiracolpus*,

it is interesting to note that despite the glacial cycles throughout the majority of *Stiracolpus*'s range of six stages and the Recent, apparent originations only occur in two of these. This suggests that the factors causing speciation are more complex than just sea level or temperature variation. Sea level variation thus might be less important in the evolution of New Zealand turritellines than for Gulf Coast species though it may be in some way correlated due to association between environmental variables. For instance, sea level change is often associated with periods of cooling, due to the formation of glaciers as in the Pliocene and Pleistocene, while other sea level changes during the New Zealand Cenozoic may be coincident with temperature variations (*e.g.*, Beu and Maxwell 1990).

Better resolved temperature and sea level curves are beginning to be produced for the Pliocene and Pleistocene of New Zealand than are available for the pre-Pliocene (*e.g.*, Carter 2005) and these, in conjunction with modern sequence stratigraphic models of these basins, should allow more detailed analyses of speciation events in *Stiracolpus,* and specifically allow tests 3 and 4 to be applied with greater accuracy. Future detailed correlation of formation-level origination data with the sequence stratigraphy and oxygen isotope stages may help to clarify why speciation does not appear to occur in *Stiracolpus* after the end of the Pliocene when examining ghost ranges while raw FAD counts indicate more gradual appearances.

DISCUSSION/CONCLUSIONS

What then can we reasonably expect the fossil record to contribute to our understanding of speciation in marine gastropods? The first answer is a failing of neontology rather than paleontology: data from modern marine gastropods are not yet adequate to provide a clear set of expectations that we can readily apply to the fossil record. The information that is available (on both cryptic species and phenotypic variation) seems to indicate that, although morphospecies based on shell form can frequently be roughly equivalent to reproductively isolated evolutionary units, caution should be used when recognizing species of marine gastropods in the fossil record. Similarly, although there is abundant evidence for allopatric speciation in modern marine gastropods, suggesting that patterns consistent with such processes can usefully be sought in the fossil record, there is also abundant evidence for non-allopatric speciation. More study is needed to determine the relative frequency of these different modes among modern forms, and whether or how non-allopatric processes might be recognizable in fossils.

If we make the assumption that allopatry is the most frequent mode of speciation in marine gastropods, the few studies that are available hint at some general patterns. For example, some recent studies of geographic range of fossil gastropods at the faunal level suggest that taxa with narrower ranges have higher rates of speciation, perhaps because those attributes that contribute to wide geographic range also suppress allopatric speciation (*e.g.*, Jablonski and Roy 2002). Many more such studies are needed to test the generality of this result. Several studies of individual clades also show a negative relationship between geographic range and origination rate (*e.g.*, Hansen 1978, 1980, Allmon 1996). Together with studies of modern species, these results suggest at least one potential solution to the "paradox of marine speciation". At least in gastropods, a combination of temporal environmental change and environmental heterogeneity can create numerous opportunities for small-scale allopatry. Environmental changes may fragment large geographic ranges and reduce local population sizes. Under these conditions, environmental heterogeneity that might be less significant within larger geographic ranges can produce and maintain barriers for sufficient time to allow genetic differentiation.

Among both paleontologists and neontologists, the assessment and use of the fossil record as a source of insight about speciation are contradictory; it is sometimes hailed as a unique source of the perspective of "deep time" and at other times assailed as so incomplete as to be essentially useless. Yet, as other authors have noted (*e.g.*, Kidwell and Flessa 1996, Behrensmeyer *et al.* 2000, Kidwell and Holland 2002, Paul 2009), the fossil record is neither entirely trustworthy nor entirely misleading. Rather, we must learn to assess the character of its data on a case-by-case basis, depending on the questions that we are asking.

Despite the substantial analytical attention that has been given to the nature and completeness of paleontological data, we are just beginning to develop and use the tools that are necessary to evaluate the record as a serious source of data about the origin of species. The tests we present here do not provide comprehensive assessments of the adequacy of any particular fossil record to inform us about speciation, but the results we have discussed here suggest that, if a single record is examined from many angles and using many kinds of information, a more comprehensive assessment of its adequacy for addressing particular evolutionary questions can be developed. The results we report here are only semi-quantitative. They can compare one record with another, to evaluate which is more reliable, but they cannot estimate with great precision the actual length of time between the EFA and FAD of a particular species, nor assess statistical significance of the patterns they reveal. These necessary aspects await further work.

The two particular fossil records of Cenozoic turritelline gastropods examined here demonstrate both the challenges and some of the opportunities inherent in the shallow-shelf marine fossil record. It is not so much the "incompleteness" of the record that limits our ability to test hypotheses and

draw conclusions, but its temporal resolution. At least one of the examples considered here is consistent with the emerging neontological hypothesis that small-scale allopatry, caused by environmental change and heterogeneity, is at least occasionally an important process in the evolution of some caenogastropod clades inhabiting shallow marine shelves. Future paleontological analyses—which take the completeness of the record adequately into account—could build on these results and begin to compile a database of relative frequencies of such environmental-evolutionary linkages in the context of explicit models of evolution, rather than just large-scale correlation. To the degree that this occurs, the fossil record could eventually become a source of significant insight about the processes of speciation.

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Change of Editors

With the publication of Volume 29, Editor-in-Chief Prof. Ken Brown and Managing Editor Dr. Cynthia Trowbridge have completed their five-year tenure as editors of the *American Malacological Bulletin*. We would sincerely like to thank all the reviewers of manuscripts, who, sometimes repeatedly, spent their valuable time helping our contributors to improve their submissions. We would also like to thank the Guest Editors who developed and often helped review the submissions for invited symposia. With Volume 30, the new editor will be:

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cwinters@towson.edu

Please join with me in welcoming Dr. Winters to the position of editor, and in wishing her success. All new manuscripts should be sent directly to Editor-in-Chief Winters.

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American Malacological Society Treasurer's Reports

Calendar Year 2008

At year end (December 31, 2008), funds available to the Treasurer were as follows:

Operating Funds		
Non-profit checking account		
Wachovia National Bank, Arlington, Virginia		$44,009.31
Endowment Funds		
Life Membership Endowment		
	$13,552.71	
Symposium and Student Research Grant Endowments		
Stock Portfolio	$41,552.59	
Bond Index Fund	$67.141.97	
TOTAL INVESTED ASSETS		$122,247.27
TOTAL ASSETS: Operating & Endowment Funds		**$166,256.58**

Total assets fell in 2008 from $206,703.21 to $166, 256.58, a decrease of $40,446.63. The decrease was due to stock market collapse driven loss in the value of investments.

Calendar Year 2009

At year end (December 31, 2009), funds available to the Treasurer were as follows:

Operating Funds		
Non-profit checking account		
Wachovia National Bank, Arlington, Virginia		$30,590.28
Endowment Funds		
Life Membership Endowment		
	$17,442.06	
Symposium and Student Research Grant Endowments		
Stock Portfolio	$53,477.35	
Bond Index Fund	$71,126.41	
TOTAL INVESTED ASSETS		$142,045.82
TOTAL ASSETS: Operating & Endowment Funds		**$172,636.10**

Total assets rose in 2009 from $166,256.58 to $172,636.10, an increase of $6,379.52.

AMS MEETING 2011
Pittsburgh, Pennsylvania, U.S.A.
23-28 July 2011

The 2011 annual meeting of the American Malacological Society will be held in Pittsburgh, Pennsylvania. Pittsburgh is home to the Carnegie Museum of Natural History (and its world class collection of freshwater and terrestrial molluscs) as well as Duquesne University, sitting on a bluff overlooking the Monongahela River and the downtown area. The meeting will be held at Duquesne University. There will be housing available in the student dorms and there is a nearby hotel for those desiring more plush accommodations. A meal plan will be available through the university.

The Presidential Symposium "Molluscs—Where are we headed" will explore recent advances in malacology as well as explore areas of current research interest. We expect to cover all classes of molluscs.

There will be sessions on gastropod behavior, life history strategies, ecology, molluscan conservation, and paleomalacology. Other sessions are being planned. We also expect to host a late afternoon roundtable discussion on conservation issues. In addition to planned sessions, there will be opportunities for presentations at open sessions as well as a poster session. The Carnegie Museum, which is a short bus trip from Duquesne University, will be available for attendees who wish to visit and use the collection.

We will have the annual auction to support student grants and a banquet. There are two field trips in the planning stages. One trip will be studying the land snail of the region while the second will be studying the freshwater molluscs of western Pennsylvania

For more information contact Charlie Sturm <csturmjr@pitt.edu>.

INFORMATION FOR CONTRIBUTORS

Scope. The *American Malacological Bulletin* is the scientific publication of the American Malacological Society and serves as an outlet for reporting notable contributions in malacological research. Manuscripts concerning any aspect of original, unpublished research, important short reports, and detailed reviews dealing with molluscs will be considered for publication.

Format. Manuscripts and illustrations should be submitted electronically (in a Word document or pdf file with embedded figures). Text must be typed in 12 pt font on 8.5 × 11 inch (letter-sized) paper, double-spaced, with all pages numbered consecutively. Leave ample margins on all sides, and left-justify the text. Final submission of accepted, revised manuscripts must include the text, tables, etc. as a mandatory **MS Word** file on a CD, DVD, or e-mail attachment, along with high resolution TIFF files of all figures. Authors should make sure all figures have at least 350 dpi resolution, and check figure files for acceptability at Sheridan's web site (dx.sheridan.com). Please follow current instructions for authors given at the AMS website or at the back of recent issues of the Bulletin.

Sections. Text, when appropriate, should be arranged in sections as follows:

1. Cover page with title, author(s), address(es), e-mail address (of corresponding author), and suggested running title of no more than 50 characters and spaces. Authors should also supply 5 key words or short phrases, placed at the base of this page, for indexing purposes. Key words or phrases should not duplicate terms already in title.
2. Abstract (less than 5% of manuscript length)
3. Text of manuscript starting with a brief introduction followed by materials and methods, results, and discussion.
   - Separate main sections of text with centered titles in bold face, capital letters.
   - Subheadings (1st level) should be left-justified and bold face; capitalize first letter.
   - Subheadings (2nd level) should be in italics with no bold face. Please refer to example below:

> MATERIALS AND METHODS
>
> **Taxonomy**
>
> **Animals**
>
> *Cultured animals*
>
> *Wild animals*
>
> **Behavioral observations**
>
> RESULTS

4. Acknowledgments
5. Literature cited
6. Figure legends (together)
7. Tables (each on a separate sheet, headed by a brief legend)

Taxonomic Authorities. All binomens, excluding non-molluscan taxa, must include the author and date attributed to that taxon the first time the name appears in the manuscript, such as *Crassostrea virginica* (Gmelin, 1791). A comma is required between the authority and date. The full generic name along with specific epithet should be written out the first time that taxon is referred to in each paragraph. The generic name can be abbreviated in the remainder of the paragraph as follows: *C. virginica*. The taxonomic authorities of generic names must be provided if species names are not included. Please refer to recent issues for examples.

Text References. Literature citations should be cited within text as follows: Hillis (1989) or (Hillis 1989). Dual authorship should be cited as follows: Yonge and Thompson (1976) or (Yonge and Thompson 1976). Multiple authors of a single article should be cited as follows: Beattie *et al.* (1980) or (Beattie *et al.* 1980).

Literature Cited Section. References must also be typed double-spaced. All authors must be fully identified and listed alphabetically; journal titles must be unabbreviated. When more than one publication with the same first author is cited, please arrange citations as follows: (a) single author, according to publication dates; (b) same author and one co-author in alphabetical, then chronological order; (c) same author and more than one co-author.

Citation Format.

Beattie, J. H., K. K. Chew, and W. K. Hershberger. 1980. Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Proceedings of the National Shellfisheries Association* **70:** 184-189.

Hillis, D. M. 1989. Genetic consequences of partial self fertilization on populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* **7:** 7-12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In:* D. C. Rhoads and R. A. Lutz, eds., *Skeletal Growth of Aquatic Organisms.* Plenum Press, New York, New York. Pp. 23-67.

Yonge, C. M. and T. E. Thompson. 1976. *Living Marine Molluscs.* William Collins Son and Co., Ltd., London.

For more detailed examples of journal series, supplements, graduate theses, governmental reports, Internet citations, non-English citations, or other categories of references, please check the following AMS web page under "Submission": http://malacological.org/publications/authors.html.

Resources. The manuscript should follow the style rules outlined in *The CSE Manual for Authors, Editors, and Publishers* (7th edition, June 2006). This can be purchased from the CSE at 12100 Sunset Hills Rd., Suite 130, Reston, Virginia 20190, USA or at the following web site: http://www.councilscienceeditors.org/publications/style.cfm. Spelling should follow American English as listed in Merriam-Webster Online (http://www.m-w.com/) or recent hardcopy editions.

Punctuation. Punctuation should follow that shown in the most recent issues of *AMB*. Specific common examples include:

- Italics: *e.g., i.e., sensu, per se, et al.*
- Non-italicized text: pers. comm., pers. obs., and unpubl. data
- Font: please use Times New Roman 12 (if possible).

Figures. Authors are strongly encouraged to submit electronic copies of the figures on CDs or DVDs (hardcopy submissions may incur additional costs). Illustrations should be clearly detailed and readily reproducible. Fine patterns and screens do not reproduce well. All figure panels must be marked with capitalized letters (A, B, C, etc . . .) and adequately labeled with sufficiently large labels to remain readable with reduction by one half. Magnification bars must appear on the figure (except for graphs), or the caption must read horizontal field width = x mm or x μm. All measurements must be in metric units. When creating figures, use font sizes and line weights that will reproduce clearly and accurately when figures are sized to the appropriate column width. Please do not include figure legends in a graphic file. Explanations of abbreviations used in a figure should occur in the legend. Please see recent journal issues for correct format. Indicate in text margins the appropriate location in which figures should appear. Color illustrations can be included at extra cost to the author (currently about $650-700/figure).

Figure Format. For final revisions of papers submitted to the editor, all electronic figure files should be in **TIFF** formats, preferably with LZW image compression. Each individual figure or graphic must be supplied as a separate, standalone file (not as an embedded object). Figure files must be named with their respective numbers and graphic type such as Fig1.tif, Figure2.tif, etc.

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Mandatory AMB Style. Any manuscript not conforming to AMB format will be returned to the author for revision before publication. Manuscripts are accepted contingent upon authors making final AMB stylistic revisions.

New Taxa. The Bulletin welcomes complete descriptions of new molluscan taxa. Establishment of new taxa must conform with the International Code of Zoological Nomenclature (1999). Descriptions of new species-level taxa must include the following information in the order as given: higher taxon designation as needed for clarity; family name with author and date; generic name with author and date; *Genus species* author sp. nov. followed by numeration of all figures and tables; complete synonymy (if any); listing of type material with holotype and any other type material clearly designated along with complete museum catalogue or accession information; listing of all additional non-type material also with full museum deposition information; type locality; diagnosis and full description of material done in telegraphic style including measurements and zoogeographic distribution as necessary; discussion; etymology. Descriptions of new supraspecific taxa should include type species (for new genus) or type genus (for new family), diagnosis and full description done in telegraphic style, and list of included taxa.

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Submission. Submit all manuscripts to: Dr. Kenneth M. Brown, *Editor-In-Chief,* Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803, USA. Please refer to the AMS web page for more detailed information prior to submission. http://malacological.org/publications/authors.html.

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